NEURAL CORRELATES OF CATEGORISATION IN THE FOREBRAIN OF PIGEONS (*COLUMBA LIVIA*)

by

Catrona Alexena Ming Chi Anderson

BA, DipLang, BA(Hons)

Supervised by Professor Michael Colombo

Submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD) in Neuroscience

Department of Psychology

Division of Sciences

University of Otago

2021

© 2021

Catrona Alexena Ming Chi Anderson

ALL RIGHTS RESERVED

To my wonderful grandma and my big bean:

you taught me strength and love

This is for you, I love you,

and I miss you both so much

ABSTRACT

The objective of this thesis is to examine neural activity in the pigeon brain during a wide range of categorisation tasks. Single-unit electrophysiology will be used to record from neurons in a prefrontal area (NCL) and an intermediary visual area (ENTO), in an attempt to understand how neurons in both of these areas are involved in the categorisation process, and how neurons from these areas use reward/behavioural information and visual information about categories during discrimination. We then present an integrated model of the neural correlates of categorisation in the pigeon brain based on our findings.

This thesis starts with an exploration into the second intermediary visual area and the termination point of the thalamofugal pathway, the visual Wulst (Chapter 3). While we found some interesting evidence of the Wulst being a highly reward-driven visual area, we decided not to continue recording from this area for the remainder of the studies as further research is needed to understand the Wulst's primary mode of processing.

The first of the categorisation tasks was a semi-symbolic categorisation task, in which birds learned to distinguish several English four-letter words from four-letter nonwords (Chapter 4). Neural activity was recorded while the pigeons discriminated their learned "vocabularies" from an extensive pool of nonwords. We found that there were no differences between activity to words and non-words, except in birds who were potentially still learning the task. We argue that behavioural information such as reward

helps guide categorisation in NCL and ENTO before learning but is likely no longer used after learning.

In the second categorisation task, we trained birds to respond to either Monet or Picasso paintings using an S+/S- paradigm (Chapter 5). We recorded neural activity from an additional area of interest known as the mesopallium ventrolaterale (MVL), a higher-order visual area. In both NCL and ENTO, we found a significant left-hemisphere dominance for both reward and visual-based categorisation, while no such asymmetry was found in MVL. Furthermore, both NCL and ENTO appear to use reward information during categorisation when there is the opportunity to (i.e. differential rewards), but MVL does not.

The third categorisation task involved more complex stimuli, in which category exemplars are comprised of four smaller stimuli (Chapter 6). Exemplars from both categories share some features (irrelevant features), while other features are unique to each category (relevant features). We found that both excitatory and inhibitory ENTO neurons use visual (colour) and behavioural (relevancy) information to aid categorisation, and we posit that these neurons relay this information onto excitatory NCL neurons. Inhibitory NCL neurons then use the information encoded and received by ENTO and excitatory NCL neurons to execute a behavioural decision.

The final categorisation task involved the same stimuli as the Monet/Picasso study in Chapter 5, but in a delayed matching-to-category (DMC) paradigm instead of the original S+/S- paradigm (Chapter 7). We present preliminary results indicating that NCL, ENTO, and MVL share information about categories with each other via reciprocal projections.

ACKNOWLEDGEMENTS

Holy shit, it's actually done. I'm sitting here at the dining table slightly in disbelief. Not at the fact that I finished it, finally, but that I made it through intact. It's been a hell of a four years, sometimes literally. I've travelled and lived overseas, had (several) breakdowns and panic attacks, made some lifelong friends, cried alone in my car, become more confident in myself and my abilities, lost people I care about, learned to love myself, struggled with mental illness, and all of it has led to this, now. While I've made it to this point and I'm proud of what I've achieved, I definitely didn't do it alone.

Mike, I really can't stress how much having you as my supervisor has helped me get to where I am now. I'm not just talking about from an academic perspective, in which you have taught me how to think critically, to design studies, how to write well, and, of course, how to become a master of Excel. You even pushed me to learn a programming language and get some work experience overseas. Even though that guidance has been invaluable, what makes you a great supervisor is the fact that you care. Your lab has been a safe place in which I was able to be myself without judgement and allowed me to come into myself. When I struggled with mental health and personal matters, you had my back and allowed me to take the time I needed to heal. I will miss having you as supervisor and listening to your funny stories and banter. Thank you so much for the last six years.

Of course, I also must give my thanks to the various members of the Colombo lab over the years. To Alysha, Kristy, Chris, Catherine, and Dylan; the coolest Coo-lombo kids and a fourth year filled with laughs and good times. You have no idea how much you all

mean to me, and how you all pulled me back from one of the darkest places I've been. To Millie, who I always looked up to, who helped me open up about mental health and made sure the lab was never dull with your witty banter. To Renelyn, Maddie, Aylin, Alina, and Jenna; a bunch of wonderful, loving women whose friendship I will always cherish. To Will, for your smart and helpful insights and thoughts, and to Max, for being yourself in a world that often tries to break us. To Hayley, one of my best friends and someone I know I can always trust, a beautiful person inside and out. To those I wasn't as close to but still appreciated nonetheless; Jess M., Jess L., Blake, Kate, Ethan, and Hayden. Colombo lab, I wish you all the best of luck for the future, and hope we meet again. Stephi, Sevim, Anna, Sara, Noemi, and John; you welcomed me as your friend and lab mate in Germany, and you all have a special place in my heart.

I wanna be defined by the things I love, not the things I hate, and your friendship, Sebi, is one defining moment I won't forget. I was terrified and *22* when I left for Germany, but you and Jutta took me under your wing without a second thought. You guys taught me how to be *Fearless* and the seven months I spent there ended up being one of the best experiences of my life. I'm not really exaggerating when I say I wouldn't survived without you guys, so *here's a toast to my real friends, rosé flowing with your chosen family*. I will always be grateful that I met you guys, and I love you both.

Speaking of chosen family: Victoria. You have put up my shit for the last nine years and you're honestly an angel for having done so. I know you think you haven't done anything in particular to help me, but that is far from the truth. You've made me laugh when I was sad, kept me sane when I was going crazy, and always, always been there when I needed you most. You are amazing, kind, beautiful, and loyal. I'm gonna miss living with you, eating snacks and watching bad reality TV. I'm gonna miss Mate cuddles

and listening to your dog mum adventures. I'm gonna miss cracking bad jokes, and you rolling your eyes at me. I will even miss your terrible Victoria stories that go nowhere. But even though we will be cities, and eventually, countries apart, you will always mean the world to me. You are my best friend, my chosen family, and always will be.

Lastly, and certainly not least, I want to thank my family. You have always supported my decisions, even when you may not have agreed with them. Mum, I aspire to have half the drive you do. You're a strong, hardworking woman, and I will kick the ass of anyone who dares to try and shame you for being the working parent. Dad, I aspire to have half the passion for learning that you do. You taught me to be curious and ask questions, and you have the biggest heart. Drew, you are the best brother a person could ask for, even when I wasn't always the best sister. You are funny, kind, and smarter than you give yourself credit for. I promise that I will always be there for you. To my extended family in New Zealand: Aunty Heather and Aunty Gael, Erin, Aroha; I love you all heaps. To my extended family overseas: Aunty Dee, Andy, Iola; Aunty Ande and Uncle Richard, Arran and Sasha; Uncle Basil and Aunty Tina, Bryan and Megan; Kyle, Isobel and Robert, the Duffs; thank you for being part of my life and I hope I see you again soon.

As a new Chapter begins, I leave you with a quote from my favourite book:

Watch carefully, the magic that occurs, when you give a person just enough comfort, to be themselves.

- *Harper Lee,* To Kill a Mockingbird

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

˚C	degrees Celcius
μm	micrometre(s)
A	arcopallium
ABNC	The Avian Brain Nomenclature Consortium
Ac	nucleus accumbens
AI	A-irrelevant
AIrrel	A-irrelevant
am	ante meridiem
ANOVA	analysis of variance
AP	anterior-posterior axis
AR	A-relevant
ARel	A-relevant
ARRIVE	Animal Research: Reporting of In Vivo Experiments
B	nucleus basalis
Bas	nucleus basorostralis pallii
BI	B-irrelevant
BIrrel	B-irrelevant
BR	B-relevant
BRel	B-relevant
C	comparison period
CA	California
Cat	Category
Cd	caudate nucleus
CDL	dorsolateral corticoid area, area corticoidea dorsolateralis
CE	excitatory neurons at the category level
CED	Cambridge Electronic Design
CI	inhibitory neurons at the category level

cm	centimetre(s)
cm^2	centimetre(s) squared
CO	common outcomes
D	delay period
DA	tractus dorso-acropallialis
dB	decibel(s)
DLP	dorsolateral posterior nucleus
DMC	delayed matching-to-category
DMS	delayed matching-to-sample
DO	differential outcomes
DR	delayed response
	discrimination ratio
DV	dorsal-ventral axis
e	excitatory
E	entopallium
Ed/Eds	editor(s)
ENTO	entopallium
FI	fixed irrelevant
GCM	generalised context model
GLd	nucleus geniculatus pars dorsalis
GP	globus pallidus
GPe	globus pallidus external segment
GPi	globus pallidus internal segment
h	height
HA	hyperstriatum accessorium, hyperpallium apicale
HD	hyperpallium densocellulare
HI	hyperpallium intercalatum
Hp	hippocampus
HV	hyperstriatum ventral
Hz	hertz
i	inhibitory

IEEE	Institute of Electrical and Electronics Engineers
IHA	interstitial hyperstriatum accessorium, nucleus interstitialis hyperpalli apicalis
Inc	Incorporated
IT	inferotemporal
ITI	intertrial interval
kg	kilogram(s)
l	length
L2	field L2
LPO	lobus parolfactorius
M	mean
	mesopallium
MA	Massachusetts
MD	mediodorsal nucleus of the thalamus
	mesopallium dorsale
MI	Monet-selective inhibitory neurons
MIT	Massachusetts Institute of Technology
ML	mediolateral axis
mg	milligram(s)
mm	millimetre(s)
mm^2	millimetre(s) squared
ms	millisecond(s)
msec	millisecond(s)
MST	medial superior temporal area
MT	middle temporal visual area
MV	mesopallium ventral
MVL	mesopallium ventrolaterale
n	not visually responsive
N	nidopallium
NCL	nidopallium caudolaterale
NEC	Nippon Electric Company

NFL	nidopallium frontolaterale
NIL	nidopallium intermediale pars lateralis
NJ	New Jersey
nRt	nucleus rotundus
ns	not significant
NUTS	No-U-turn sampling
NY	New York
OB	olfactory bulb
PE	Picasso-selective excitatory neurons
PFC	prefrontal cortex
PI	Picasso-selective inhibitory neurons
PLOS	Public Library of Science
pm	post meridiem
PNAS	Proceedings of the National Academy of Sciences
pt	point
Pt	putamen
RI	inhibitory neurons at the relevance level
Rt	nucleus rotundus
s	second(s)
S	sample period
S+	positive stimulus
S-	negative stimulus
SD	standard deviation
SE	standard error
sec	second(s)
SEM	standard error of the mean
SI	inhibitory neurons at the stimulus level
SMC	simultaneous matching-to-category
sp	spikes
StM	striatum mediale
TeO	tectum opticum

TPO	area temporo-parieto-occipitalis
TuO	olfactory tubercule
UK	United Kingdom
USA	United States of America
V	volt(s)
V1	visual area 1
V2	visual area 2
V4	visual area 4
VI	variable irrelevant
w	width
WA	Washington
WM	working memory

CHAPTER 1 |

GENERAL INTRODUCTION

Categorisation is a fundamental process used to organise and simplify incoming information from the environment around us (Fabre-Thorpe, 2003). For example, it is through categorisation that we are able to recognise that both an apple and an orange are food but that a tennis ball is not food, despite all three items sharing similar properties, i.e., shape. The category of 'food' is an abstract category as it contains a wide variety of items that greatly differ in their physical properties (see Figure 1-1a). However, the category of 'apples' is a far more concrete category as all types of apples share many similar visual and physical properties (see Figure 1-1b). Being able to group objects and items by their function, and ignoring extraneous information, is an important part of cognition (Cromer, Roy, & Miller, 2010). Consequently, categorisation has been extensively studied in humans, and it is widely recognised that categorisation is comprised of multiple learning systems (Ashby & Ell, 2001; Ashby & Maddox, 2011; Gluck, Poldrack, & Kéri, 2008; Smith, Ashby, Berg, Murphy, Spiering, Cook, & Grace, 2011; Smith & Grossman, 2008). It should be noted that the ability to group familiar objects into categories is known as discrimination, and while a core component of

categorisation, true categorisation requires the ability to apply this discrimination to new objects.

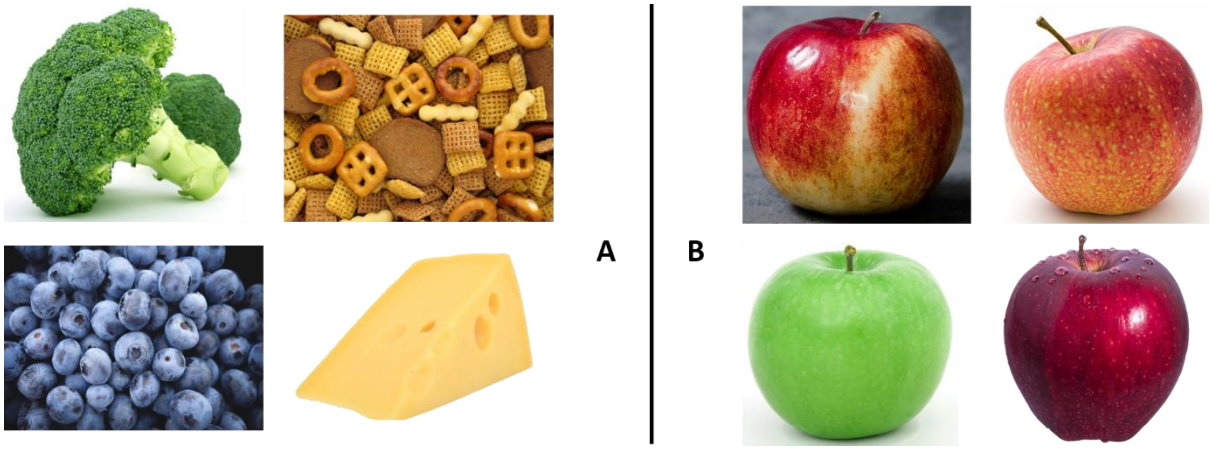

Figure 1-1: Abstract and concrete categories.

An example of an abstract category (a) and a concrete category (b). The category of 'food' is abstract because members of the category often do not share similar visual and physical properties but do share a similar function – nourishment. The category of 'apples' is concrete because all members of the category have highly similar visual and physical properties.

At a higher-order level, categories can be learned by applying rules and hypotheses about category membership to a new item within working memory (explicit system; Ashby & Maddox, 2011; Smith et al., 2011; Smith & Grossman, 2008). On a lower level, categories can also be learned through simple behaviour-perception associations, which are strengthened with reinforcement (implicit system; Ashby & Maddox, 2011; Gluck et al., 2008; Smith et al., 2011). In humans, the explicit system has been shown to encompass a broad neural network between the prefrontal cortex, anterior cingulate gyrus, the hippocampus, and the head of the caudate nucleus (Ashby & Maddox, 2011), similar to

the neural network involved in the executive control of attention (Rossi, Pessoa, Desimone, & Ungerleider, 2009). On the other hand, the implicit system appears to rely on the striatum (Smith et al., 2011), in particular, at cortical-striatal synapses (Ashby & Maddox, 2011).

The ability to categorise has previously thought to have been either restricted to humans (Herrnstein, 1984) or to chimpanzees exposed to language-training (Premack, 1983), as many categories are defined by ideas, words, and concepts in human language (Lea, 1984). However, many other non-human animals have since shown the ability to categorise information. For animals, categorisation is highly important on an ecological level, as it allows animals to recognise food from non-foods, as well as identifying potential predators. As such, some concepts or categories are not reliant on human language. Wild vervet monkeys are known to produce unique alarm calls for different types of predators, which in turns elicits different behavioural responses from surrounding monkeys (Seyfarth, Cheney, & Marler, 1980). That is, if a predator is categorised as being a leopard, alarm calls and responses will be different than if a predator is categorised as being an eagle.

In experimental studies, non-human primates can categorise various objects. For example, apes and monkeys are able to distinguish pictures containing humans from pictures without humans (D'Amato & Van Sant, 1998; Schrier & Brady, 1987), animals versus non-animals (Roberts & Mazmanian, 1988; Vonk, Jett, Mosteller, & Galvan, 2013), food versus non-food objects (Fabre-Thorpe, 2003; Fabre-Thorpe, Richard, & Thorpe, 1998), as well as complex visual images (Vogels, 1999) and morph images (Cromer et al., 2010; Freedman, Riesenhuber, Poggio, & Miller, 2001).

Non-primates such as rats also can categorise arrays of visual stimuli (Wasserman, Castro, & Freedman, 2012) as well as complex acoustic stimuli (Mercado, Orduña, & Nowak, 2005). Larger carnivores, such as dogs, are able to discriminate pictures of dogs from landscapes (Range, Aust, Steurer, & Huber, 2008), and black bears are able to categorise animals versus non animals (Vonk, Jett, & Mosteller, 2012). Even bees have the capacity to categorise natural objects such as flowers, plants, and landscapes (Zhang, Srinivasan, Zhu, & Wong, 2004). The ability to categorise information seems like a fundamental capacity across a range of different animals. For this thesis, the focus will be the categorical abilities of birds, and in particular, pigeons, which will be discussed in the following section.

1.1. The Categorical Abilities of Pigeons

Herrnstein and Loveland (1964) conducted one of the first studies investigating categorisation in animals in which pigeons were trained to discriminate between photos that contained a human versus photos without a human. Pigeons were not only able to categorise familiar human photos (that is, the ones they were originally trained with), but also generalise this ability to novel human photos. Furthermore, when pictures of humans were pasted into familiar non-human photos, pigeons were still able to categorise these as human photos, indicating that they were discriminating based on whether the photos included humans and not by the fact that certain backgrounds were associated with humans (Aust & Huber, 2001). In a similar experiment, Herrnstein (1979) demonstrated that pigeons could discriminate between photos containing trees and photos without trees. Other studies have also demonstrated that pigeons can easily discriminate various natural objects (Bhatt, Wasserman, Reynolds, & Knauss, 1988; Cook, Wright, & Drachman,

2013; Wasserman, Kiedinger, & Bhatt, 1988), as well as man-made objects (Bhatt et al., 1988; Morgan, Fitch, Holman, & Lea, 1976) and even multidimensional sine wave gratings based on frequency or orientation (Berg & Grace, 2011).

A few studies have also examined whether pigeons are able transfer to altered or novel stimuli, which are usually created by changing or deleting single features of the original stimuli. Aust and Huber (2002) trained pigeons to discriminate photos of humans and photos without humans as in Herrnstein and Loveland (1964), and then tested pigeons with new images that contained only some human or human-like features. For example, transfer stimuli included pictures with small humans, human silhouettes, individual body parts, clothing, primates and other animals, or 'pseudohumans', which were objects that resembled people such as dolls (see Figure 1-2).

Figure 1-2: Transfer stimuli used in Aust and Huber (2002).

The transfer stimuli from Aust and Huber (2002) that were used after pigeons were trained to discriminate photos with humans from photos without humans. Pigeons were tested with these stimuli to understand if pigeons were basing their original discriminations on singular local features or global features. Clothes isol, clothes isolated; Pseudoh, pseudohumans. Adapted from Aust and Huber (2002).[1]

Aust and Huber (2002) found that pigeons did not classify small humans and silhouettes as indicative of people being present in the picture, but pseudohumans and

[1] Adapted by permission from Springer Nature: Animal Learning and Behavior, Target-defining features in a "people-present/people-absent" discrimination task by pigeons, Aust & Huber, Copyright 2002.

animals wearing clothing did trigger a person-present response. However, animals on their own were not categorised as people-present. Interestingly, certain individual body parts such as hands did trigger the people-present response, yet other parts such as feet and skin did not. Other studies have demonstrated a similar effect in which certain partial features of a stimulus can often trigger a categorisation response from pigeons, such as parts of the letter A and number 2 in an A versus 2 discrimination (Morgan et al., 1976), and isolated facial features in a happy versus angry human faces discrimination (Jitsumori, & Yoshihara, 1997).

Since pigeons are able to transfer to either singular local features of stimuli, or stimuli missing some local features, it is widely recognised that pigeon discrimination must be on the basis of several local features, rather than perceiving the stimulus as a whole (Aust & Huber, 2002; Ghosh, Lea, & Noury, 2004; Jitsumori & Yoshihara, 1997; Morgan et al., 1976). That is, pigeons appear to categorise objects based on the proportion of elements the new object shares with previously learned exemplars from a category, which some researchers refer to as a Common Elements Model (Soto & Wasserman, 2010; 2012).

1.2. The Avian Brain

For this thesis, the aim is to explore neural activity via single-unit electrophysiology in pigeons during a wide range of discrimination tasks. Considerable advances have been made in the last 10 years concerning the evolution and development of the avian brain. Following a classical view of telencephalic evolution, vertebrate brains were thought to develop in a progressive and unilinear fashion from lower to higher forms of intelligence

(The Avian Brain Nomenclature Consortium [ABNC], 2005). That is, avian brains were thought to be a primitive or underdeveloped version of the human brain. Comparisons with human and mammalian brains led researchers to believe that the avian brain had evolved a large basal ganglia subdivision: in essence, a hypertrophied striatum (Güntürkün, 2005b). The avian striatum was thought to consist of three main subdivisions: paleo-, archi-, and neostriatum (ABNC, 2005). The striatum in the mammalian brains is not usually associated with cognition or cognitive abilities, and historically this fit with the view that the cognitive abilities of birds was limited.

More recent evidence, however, indicates that birds do not differ qualitatively from mammals and primates in terms of cognitive capabilities, and that previously conceived differences between these species can be traced back to confounding contextual variables (for a review, see Colombo & Scarf, 2020). Scrub-jays have displayed episodic memory (Clayton & Dickinson, 1998), a facet of theory of mind previously thought to only be present in humans, and crows can make tools to retrieve food (Weir, Chappell, & Kacelnik, 2002). Pigeons in particular have demonstrated cognitive abilities such as memorising visual patterns (von Fersen & Delius, 1989), understand abstract numerical reasoning (Scarf, Hayne, & Colombo, 2011) and using transitive logic (von Fersen, Wynne, Delius, & Staddon, 1991).

Furthermore, research has shown that the brain of birds is more conserved than previously thought (Güntürkün, 2005b) and is actually comprised of many pallial regions that resemble mammalian neocortex (Güntürkün, 2005a). Based on molecular embryology and the prevalence of dopaminergic pathways, the ABNC (2005) proposed that the classical view of the avian brain consisting of three striatal regions be re-

examined. Instead, the ABNC (2005) posited that the avian brain consists of four pallial regions that are homologous to mammalian pallial regions (see Figure 1-3).

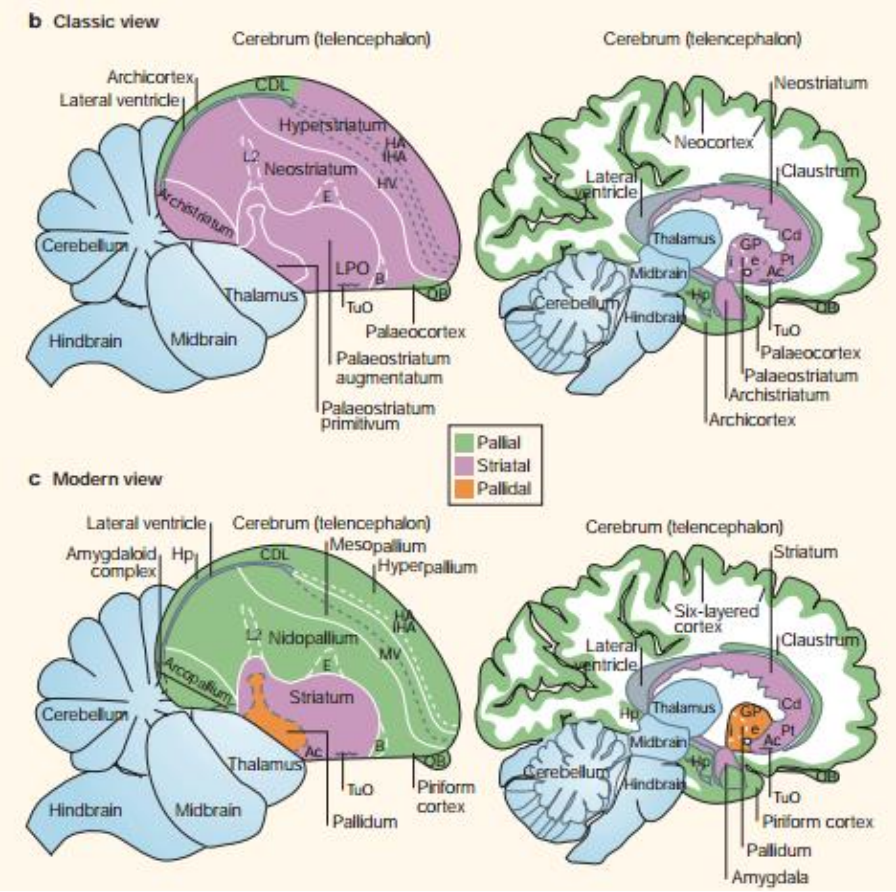

Figure 1-3: Illustration of the avian and mammalian brains.

The classical and modern view of the avian brain (left side) compared to a mammalian brain (right side). Ac, nucleus accumbens (*nucleus striae terminalis lateralis*); B, nucleus basalis (*basorostralis*); Cd, caudate nucleus; CDL, dorsolateral corticoid area; E, entopallium; GP, globus pallidus (i, internal segment; e, external segment); HA, hyperstriatum accessorium (*hyperpallium apicale*); Hp, hippocampus; HV, hyperstriatum ventrale (*mesopallium ventrale*); IHA; interstitial hyperstriatum accessorium (*interstitial hyperpallium apicale*); L2, field L2; LPO, lobus parolfactorius (*medial striatum*); MV, mesopallium ventral; OB, olfactory bulb; Pt, putamen; TuO, olfactory tubercle. From ABNC (2005)[2]. Italicised names are revised nomenclature as defined by Reiner et al. (2004).

[2] Reprinted by permission from Springer Nature: Nature Reviews Neuroscience, Avian brains and a new understanding of vertebrate brain evolution, Jarvis, E. D. et al., Copyright 2005.

Over the past decade, birds have become a popular non-human animal model for vision due to their highly evolved visual systems and abilities. Some avian brains have been shown to contain around twice as many neurons as mammalian brains of the same size (Olkowicz, Kocourek, Lučan, Porteš, Fitch, Herculano-Houzel, & Němec, 2016), and avian eyes are large compared to the size of their body (Cook, 2000), both which lead to the avian brain being highly specialised and efficient for processing visual information. Indeed, birds are able to detect more colours than humans and perceive images at higher resolutions (Cook, 2000), and can detect subtle luminosity differences that humans cannot (Hodos, Bessette, Macko, & Weiss, 1985).

This thesis will focus on two forebrain areas of the pigeon brain: the nidopallium caudolaterale (NCL) and the entopallium (ENTO).

1.2.1. Anatomy and architecture of the nidopallium caudolaterale (NCL)

The nidopallium caudolaterale (NCL) is a region in the dorsolateral forebrain that is considered to be analogous to mammalian neocortex (Rehkämper & Zilles, 1991), specifically, the prefrontal cortex (PFC; Güntürkün, 2005a; 2005b). Both the PFC and NCL are one of the main termination areas for dopaminergic fibres in the brain (Divac & Mogensen, 1985) which are highly myelinated (Rehkämper & Zilles, 1991). Furthermore, like the PFC, NCL has similar reciprocal connections to motor areas, primary and secondary sensory areas, and the amygdala (Güntürkün, 2005b; Kröner & Güntürkün, 1999). However, one difference is that the PFC receives afferent projections from the mediodorsal (MD) nucleus of the thalamus, whereas NCL receives projections from the dorsolateral posterior (DLP) nucleus – which while not homologous areas (Kröner & Güntürkün, 1999), both MD and DLP fulfil similar functions (Güntürkün, 1997).

1.2.2. The function of the NCL

Evidence of the functional similarities between the PFC and NCL come from lesion and behavioural studies which require animals to retain information to later guide behaviour. Many studies have demonstrated that lesions to the mammalian PFC are detrimental to an animal's ability to hold information during a delay period, indicating that the PFC is involved in working memory (WM; Dunnett, Nathwani, & Brasted, 1999; Jacobsen, 1936; see Curtis & D'Esposito, 2004 for a review). Similarly, lesions to the avian NCL disrupts delay performance (Gagliardo, Bonadonna, & Divac, 1996; Güntürkün, 1997), with the effect of the lesion on working memory being proportional to the size of the lesion (Diekamp, Gagliardo, & Güntürkün, 2002). Evidence from single-unit electrophysiology also shows that avian NCL neurons hold information within working memory during a delay (Johnston, Anderson, & Colombo, 2017a; Veit, Hartmann, & Nieder, 2014).

Other functions of the NCL include executive control. Rose and Colombo (2005) found that when birds were instructed to remember information (via a high-frequency tone) presented to them before a delay period, NCL neurons displayed sustained activation during the delay. When birds were instructed to forget the information (via a low-frequency tone) presented before the delay period, NCL neurons did not display the same sustained activation. Thus, it appears that NCL neurons are involved in the control of selectively remembering or forgetting information.

However, when given the opportunity to code reward-related information, NCL neurons appear to default to coding this type of information. In a similar study to Rose and Colombo (2005), Browning, Overmier, and Colombo (2011) instructed birds to either remember or forget information presented before a delay period, but reward was only

given following the presentation of one stimulus (red dot) and not the other (white dot). Browning et al. (2011) found that introducing differential reward outcomes changed the role of NCL neurons from remembering/forgetting information during the delay, to coding whether a reward would be given. Other studies have also shown that NCL neurons will default to reward coding over coding to-be-remembered information during a delay if there is the opportunity to do so (Johnston, Anderson & Colombo, 2017b). Similarly, mammalian PFC is involved in reward-related processing, especially when reward is used to help guide learning (Izquierdo, Suda, & Murray, 2004; Ono, Nishino, Fukuda, Sasaki, & Nishijo, 1984; Tzschentke, 2000).

1.2.3. The role of PFC and NCL in categorisation

In primates, PFC activity has been implicated in categorical processing. Freedman et al. (2001; 2003) trained rhesus monkeys to categorise sets of cat/dog morphed images into the 'cat' or 'dog' category based on which animal made up the largest proportion of the morphed image. Freedman et al. (2001; 2003) found that PFC activity differentiated between the cat and dog categories, but also that activity within each category was similar, indicating that the PFC is involved in the categorical representation of information (for a more comprehensive review of morph studies, see section 7.1.1.).

A few studies have examined the neural basis of categorisation in the avian NCL. In crows trained to discriminate between small and large numerosities, individual NCL neurons tend to selectively fire to a 'preferred numerosity' (Ditz & Nieder, 2015; 2016). Furthermore, the firing of these numerosity-selective neurons is proportionate to how close a particular numerosity is to their preferred numerosity. That is, if a particular neuron's preferred numerosity is five, that neuron will fire at a lower rate to other numerosities close to five (i.e. four and six), but not at all to numerosities far from five

(i.e. one and nine), indicating that NCL neurons appear to categorise numerical quantities on a gradient, rather than in absolute numbers. Another study by Veit, Pidpruzhnykova, and Nieder (2017) trained crows on a delayed paired association task, in which novel stimuli were arbitrarily assigned to one of two previously learned categories (red and blue choice keys). While discriminating between familiar (learned) stimuli, NCL neurons showed selectivity towards the behavioural choices, that is, some neurons preferred the red category, while others preferred the blue category. When presented with a novel stimulus (new learning), the same choice-selective neurons would only fire if the novel stimulus belonged to the category the choice-selective neuron preferred. That is, when assigning a new stimulus to a learned category, NCL neural activity seems to encode associations based their behavioural meaning or outcomes.

To our knowledge, only two studies have examined the role of the pigeon NCL in categorisation. In one study, Kirsch, Vlachos, Hausmann, Rose, Yim, Aertsen, and Güntürkün (2009) trained pigeons on a go/nogo task, in which mandibulation responses to a lightning or heart stimulus (go stimuli/category) and withholding mandibulation for a triangle or cross stimulus (nogo stimuli/category) resulted in reward (Figure 1-4). Kirsch et al. (2009) found that during the early stages of learning the categories, NCL neural activity occurred just before and during the reward period, indicating that these neurons were coding rewards associated with similar behavioural outcomes. That is, rewards given for stimuli that required mandibulation responses (go category) were coded differently from rewards given for stimuli that required withholding mandibulation (nogo category). However, once birds had learned the task, NCL neural activity discriminated between go/nogo stimuli from the onset of presentation and through the reward period, indicating that NCL neurons were now associating rewards with the stimuli themselves,

rather than just the behavioural response required to obtain reward. On a behavioural level, the changes in how NCL neurons are activated as categories are learned may suggest that reward is highly important for learning the category boundaries initially, but then once the categories are learned, the stimuli themselves are what drives categorisation.

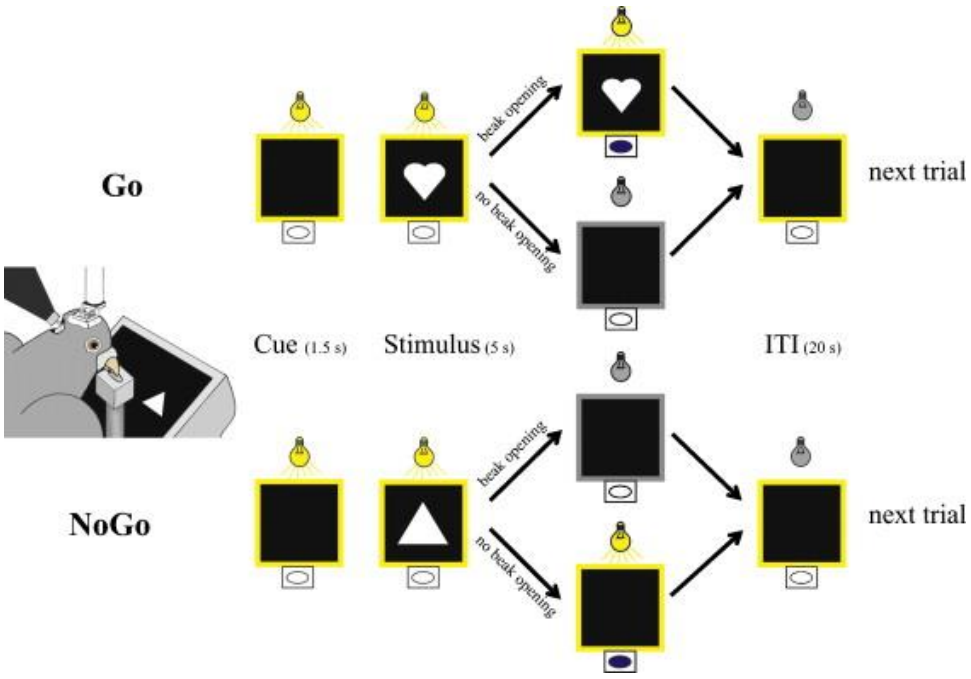

Figure 1-4: The behavioural task from Kirsch et al. (2009).

During the stimulus period, if the heart or lightning stimulus was presented, birds had to open their beak (mandibulate) in order to receive 1.5 s access to water. If the triangle or cross stimulus was presented, birds were required to refrain from mandibulating in order to receive 1.5 s access to water. Incorrect responses to any stimulus resulted in a 3 s time-out punishment. Adapted from Kirsch et al. (2009).[3]

[3] Reprinted from Behavioural Brain Research, 198, Kirsch, J. A., Vlachos, I., Hausmann, M., Rose, J., Yim, M. Y., Aertsen, A., & Güntürkün, O., Neuronal encoding of meaning: Establishing category-selective response patterns in the avian 'prefrontal cortex', 214-223, Copyright 2009, with permission from Elsevier.

Lengersdorf, Pusch, Güntürkün, and Stüttgen (2014) also examined NCL activity during a categorisation task which required pigeons to identify whether the luminance of a grey stimulus was 'dark' or 'bright'. Pigeons were trained with six different shades of grey stimuli, with the lighter three belonging to the 'bright' category, and the darker three belonging to the 'dark' category. If a pigeon was presented with a bright grey stimulus, they were required to peck the left choice key, and the right choice key if the stimulus was dark grey. Lengersdorf et al. (2014) found that NCL neural activity discriminated between the bright and dark categories during presentation, but that this distinction seemed to be based on the motor response associated with the category, rather than the luminance of the stimulus, similar to neurons in Kirsch et al. (2009) during learning. Furthermore, some NCL neurons displayed differential firing to expected rewards and unexpected rewards, which suggests that NCL neurons encodes information about reward that extends beyond whether reward simply exists or not. Thus, NCL neurons appear to use information about the reward to help guide learning, especially in categorisation tasks.

1.2.4. Anatomy and architecture of the entopallium (ENTO)

The avian visual system is comprised of two main pathways (Figure 1-5). The *thalamofugal* pathway runs from the retina through the nucleus geniculatus pars dorsalis (GLd) and ends in the telencephalon in an area known as the Wulst, and is thought to be analogous to the mammalian *geniculostriate* pathway (Shimizu & Karten, 1993). The second and main pathway is the *tectofugal* pathway, which runs from the retina to the optic tectum, then the nucleus rotundus, and ends in the telencephalon in the area known as the entopallium (ENTO), which is considered to be analogous to the mammalian *colliculothalamocortical* pathway (Shimizu & Karten, 1993). ENTO has been compared to some portion of primate extrastriate cortex (Johnston & Colombo, 2017), as both

ENTO and inferior temporal (IT) cortex in primates have little to retinotopic mapping, and have neurons with large receptive fields (Gross, Rocha-Miranda, & Bender, 1972; Gross, Rodman, Gochin, & Colombo, 1993; Gu, Wamg, Zhang, & Wang, 2002). However, projections between the nucleus rotundus and entopallium are topographically organised along the anterior-posterior axis, as well as projections from the entopallium to other pallial areas (Johnston & Colombo, 2017), including minor projections to NCL (Krützfeldt & Wild, 2005).

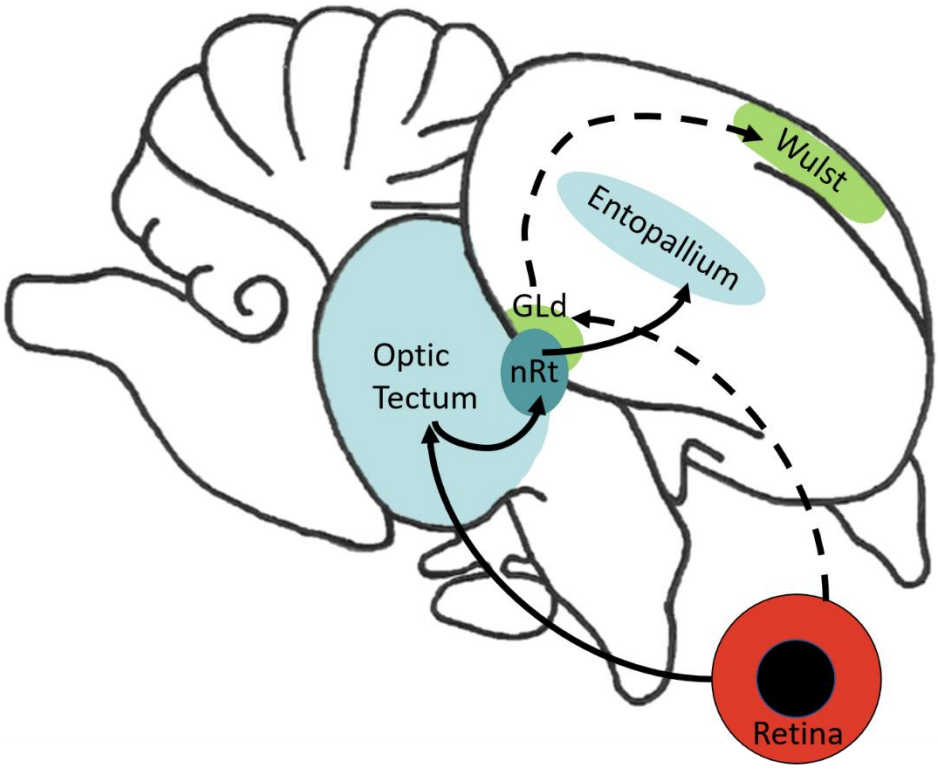

Figure 1-5: The two pathways of the avian visual system.

The tectofugal pathway (solid arrows) runs from the retina to the optic tectum and the nucleus rotundus, terminating at the entopallium. The thalamofugal pathway (dashed arrows) runs from the retina to the nucleus geniculatus pars dorsalis, terminating in the Wulst. GLd, nucleus geniculatus pars dorsalis; nRt, nucleus rotundus.

1.2.5. The function of the ENTO

While ENTO and primate extrastriate cortex are comparatively similar in anatomy, they are also similar in function. Several lesion studies have shown that ablations to ENTO and the tectofugal pathway cause severe deficits in perception, particularly in the ability to discriminate luminance and patterns (Bessette & Hodos, 1989; Hodos & Karten, 1970; Hodos, Weiss, & Bessette, 1988;), and motion (Nguyen, Spetch, Crowder, Winship, Hurd, & Wylie, 2004). Similar deficits occur in extrastriate cortex association areas. Damage to area MT in primates causes deficits in motion processing (Pasternak & Merigan, 1994; Rudolph & Pasternak, 1999), damage to area V4 causes deficits in luminance discrimination (Heywood & Cowey, 1987; Walsh, Carden, Butler, & Kulikowski, 1993) while damage to IT cortex causes deficits in pattern processing (Butter & Gekoski, 1966; Cowey & Weiskrantz, 1967).

It is clear that ENTO represents some intermediary visual area in the avian brain and is responsible for many visuo-perceptual functions. However, there is also evidence that ENTO has involvement in visual memory. ENTO neurons display sustained firing during the delay period of matching-to-sample tasks, indicating that these neurons are holding visual information to guide later behaviour (Colombo, Frost, & Steedman, 2001; Johnston et al., 2017a). Interestingly, under differential outcomes, the visual information held by ENTO neurons over a delay period is modulated to some extent by reward information (Johnston et al., 2017a).

1.2.6. The role of extrastriate cortex and ENTO in categorisation

In primates, parts of extrastriate cortex (in particular, the IT cortex) have also been implicated in categorical processing. In the same study described in section 1.2.3. by Freedman et al. (2003), IT cortex neurons were also recorded from during the cat/dog

morph categorisation task. Unlike PFC activity, IT cortex activity appeared to be more reactive to specific stimulus features, rather than category membership or behavioural outcomes. Sigala and Logothetis (2002) also found that IT cortex neurons focus on specific stimulus features, particularly those that are indicative of category membership (i.e., features that are unique to a category).

A number of studies have examined the effects of ENTO lesions on the categorisation ability of pigeons. Watanabe (1991) trained pigeons on various visual discrimination tasks, including food versus non-food objects, and a pseudo-discrimination version where food and non-food items were arbitrarily assigned to one of two categories. Watanabe (1991) found that bilateral ENTO lesions specifically caused deficits on the pseudo-discrimination task, but not on the natural concept of food versus non-food. In a later study, Watanabe (1993) found the same impairment on food versus non-food discrimination when ENTO was lesioned. However, when trained on other natural concept tasks that required pigeons to discriminate between individual pigeons and pigeons versus other birds, Watanabe (1992) found that ENTO lesions only caused specific deficits in the ability to discriminate between individual pigeons, but not in the ability to discriminate between pigeons and other bird species. Watanabe (1992; 1996) argues that ENTO lesions cause a "category-specific agnosia", particularly to categories that are not ecologically important to pigeons or are new (unlearned) categories. On the other hand, categories that are important for survival such as food items are not affected by ENTO lesions. Thus, ENTO's involvement in categorisation appears to be stronger during learning, when differences in visual features are important for establishing category boundaries.

To our knowledge, only two studies have examined single-unit activity in ENTO during a categorisation task. Scarf, Stuart, Johnston, and Colombo (2016) examined ENTO neural activity while passively viewing different stimuli which included three images of pigeons. Although there was no behavioural requirement to categorise the stimuli, other studies have shown that neurons still naturally categorise stimuli to some degree while passively viewing (in humans and non-human primates, see Kriegeskorte, Mur, Ruff, Kiani, Bodurka, Esteky, Tanaka & Bandettini, 2008; in pigeons see Koenen, Pusch, Bröker, Thiele, & Güntürkün, 2016). However, Scarf et al. (2016) failed to find any ENTO neurons that fired preferentially for the pigeon stimuli, that is, no ENTO neurons seemed to categorise pigeon stimuli as any different to shapes, colours, or even a picture of Paddington Bear. Another study by Clark, Porter, and Colombo (2019) also failed to find evidence for pigeon face cells being represented in ENTO. However, in both Scarf et al. (2016) and Clark et al. (2019), it is possible that a lack of category representation in ENTO is due to the fact that pigeons are predisposed to focusing on local features over global features. Thus, ENTO is likely still involved in other categorisation tasks which allow pigeons to use local features to discriminate categories, and any involvement in categorisation should not be discounted as indicated by the aforementioned lesion studies.

1.3. Aims and Objectives of the Thesis

The objective of this thesis is to examine neural activity in the pigeon brain during a wide range of categorisation tasks that are based on previous behavioural studies (Chapters 3-6). Single-unit electrophysiology will be used to record from neurons in a prefrontal area (NCL) and an intermediary visual area (ENTO), in an attempt to

understand how neurons in both of these areas are involved in the categorisation process, and how neurons from these areas use reward/behaviour-driven information and visual information about a category during discrimination.

This thesis will start first with an overview of the general methods and analyses used across all five studies (Chapter 2). The first study will be an exploration into another intermediary visual area and the termination point of the thalamofugal pathway, the visual Wulst (Chapter 3). Chapter 3 will use a delayed matching-to-sample (DMS) paradigm while recording from the Wulst in order to understand its involvement in working memory. Data from the Wulst DMS study will then inform us whether the Wulst will be used as an area of interest alongside NCL and ENTO for subsequent categorisation studies (Chapters 4-7), the main focus of this thesis.

The first of the categorisation tasks will be a semi-symbolic categorisation task, in which birds have learned to distinguish several English four-letter words from four-letter nonwords (Chapter 4). Neural activity will be recorded while the pigeons discriminate their "vocabularies" from an extensive pool of nonwords. In the second categorisation task, we trained birds to respond to either Monet or Picasso paintings using an S+/S- paradigm (Chapter 5). In the Monet/Picasso study, we recorded neural activity from an additional area of interest known as the mesopallium ventrolaterale (MVL), a higher-order visual area upstream from both ENTO and the Wulst. The third categorisation task involves more complex stimuli, in which category exemplars are comprised of four smaller stimuli. Exemplars from both categories share some features, while other features are unique to each category (Chapter 6). The final categorisation task will involve the same stimuli as the Monet/Picasso study in Chapter 5, but in a delayed matching-to-category (DMC) paradigm instead of the original S+/S- paradigm (Chapter 7).

CHAPTER 2 | GENERAL METHODS

In this Chapter, an overview of the methods and analyses common to all of the studies within this thesis is given. Any variations to the common methods and analyses are outlined within individual Chapters.

2.1. Subjects

Pigeons were housed individually in a colony room in cages made of thick wire mesh (50 mm long and 30 mm wide holes) measuring approximately 50 cm^2 each for the four sides, with a solid ceiling and floor made of sheet metal. The front-facing side of each cage had a hinged door. All cages were padded with newspaper on the bottom and were provided with a wooden perch suspended above the floor, which locked into place through the holes of the mesh. The colony room was maintained at 20 °C and with a light/dark cycle of 12 hours (7am–7pm). Water and grit were always available. The birds were fed a mixture of peas, corn, wheat, and sunflower seeds, and kept within 80–85% of their free-feeding weight during training and testing. The animals were handled and cared for in accordance with the University of Otago Code of Ethical Conduct for the Manipulation of Animals. The ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010) were also assessed in order to improve the reporting of this research.

2.2. Apparatus and Stimuli

Two operant chambers measuring 350 (l) x 430 (w) x 390 (h) mm internally was used for training and electrophysiological testing. At the front of each chamber was a 17-inch monitor that was used to display the stimuli. Perspex panels were positioned in front of the monitors (see each Chapter for specifications on the Perspex panels for each study). Carroll Touch infrared touch frames (EloTouch) were positioned between the monitors and the Perspex panels. The touch frames recorded the XY coordinates of all pecks made by the birds. Food hoppers with grain were located 110 mm below the lower middle square and rose to floor level to reward the birds. A light located by the hoppers was illuminated while reward was being delivered.

2.3. Microdrive Surgery

For surgery, the birds were injected with a mixture of Ketamine (30 mg/kg) and Xylazine (6 mg/kg). The feathers on the head were removed and the pigeons were placed in a Revzin stereotaxic adapter (Karten & Hodos, 1967) to immobilise the head. A topical anaesthetic (10% Xylocaine) was applied to the scalp before the skin over the scalp was retracted to expose the skull. Seven stainless steel screws were inserted into the skull, with one serving as the ground screw. A hole was drilled into the skull above the area of interest, and the dura was removed. A lightweight microdrive (Bilkey, Russell, & Colombo, 2003) housing the electrodes was lowered into the hole until the tips of the electrodes were positioned directly above the area of interest. The microdrive was secured to the skull using dental acrylic, and the wound was sutured. Xylocaine was reapplied to the wound margin and the pigeons were placed in a heated recovery cage. The pigeons remained in the recovery cage until they returned to an active state, at which point they

were administered an analgesic (Carprieve, 5 mg/kg), and returned to their home cages. The birds were given another seven days to recover before testing began.

For NCL birds, the electrodes were positioned at AP +5.5, ML ±7.5, DV = 1.0. For ENTO birds, the electrodes were positioned at AP +9.5, ML ±6.0, DV = 3.0 (see each Chapter for specific co-ordinates and placements of the microdrives).

2.4. Neuronal Recording

Each microdrive contained eight 25 µm Formvar-coated nichrome wires (California Fine Wire, Grover Beach, CA, USA) which were used to measure single-unit activity. Before each testing session, we searched for activity on any one of the eight wires, using a second wire as the indifferent. The activity was amplified using a Grass P511K (Grass Instruments, Quincy, MA, USA) and filtered to remove 50 Hz noise. The data was stored and analysed by a CED (Cambridge Electronic Design, Cambridge, UK) electrophysiology system with Spike2 software. A separate computer controlled the behavioural task and sent codes to the CED system to align key task events.

After each session, if we recorded single-unit activity, the electrodes were advanced approximately 40 µm and the pigeons were returned to their home cages. If we did not record single-unit activity during a session, then the electrodes were advanced approximately 20 µm and the pigeons were returned to their home cages. Recording sessions took approximately one hour to complete, and the pigeons completed one session daily, five days per week.

2.5. Neural Analysis

As with our previous studies (Johnston et al., 2017a; 2017b), we took baseline activity as the middle 5 s of the 10 s intertrial interval (ITI). To measure whether a neuron fired to the sample stimulus, we could use either of two time points: the 'stimulus first' period, i.e., a period of time immediately after the sample stimulus was presented; or the 'stimulus last' period, i.e. a period of time immediately before the first peck to a sample stimulus. For the 'stimulus first' period, the 300 ms stimulus period was taken from +100 ms after stimulus onset until +400 ms. We start the 'stimulus first' period at +100ms to avoid the possibility that stimulus information had not yet been processed. For the 'stimulus last' period, the 300 ms period is taken from –400 ms to –100 ms prior to the first of the required pecks to the sample stimulus. We omit the last 100 ms prior to the peck because pigeons are known to close their eyes up to 100 ms prior to making contact with an object (Goodale, 1983). While most of our studies use the 'stimulus last' period, we had to use the 'stimulus first' period in our S+/S- discrimination (see Chapter 5).

2.6. Histology and Electrode Track Reconstruction

When the electrodes reached the end of the area of interest, a 9 V current was sent through each electrode for 10 s to create an electrolytic lesion, marking the final recording position. The pigeons were euthanised via carbon dioxide gas, and then perfused with physiological saline and 10% formalin. The brains were removed from the skull and kept in 10% formalin for at least 5 days, followed by sucrose formalin (10% formalin, 30% sucrose). The brains were frozen and sliced into 40 µm sections, which were then stained with thionin. Track reconstructions were made using the position of the electrolytic lesion and depth records.

2.7. Data Analysis and Filtering for Visually Responsive Cells

There are two ways in which neural activity can be compared. One method is to statistically compare the neural activity during one set of trials to the neural activity during another set of trials. The limitation of such an analysis is that it is possible that one may differ from the other, yet neither would actually differ from baseline levels of activity. Rather, and because of the aforementioned limitation, neurons were categorised as visually responsive if they displayed activity during the sample period that was significantly different from baseline ITI activity, for trials containing one set of stimuli, or both sets. Such a method ensures that any differences in activity between trial types are not due to chance.

Neurons with a firing rate of less than 0.2 Hz during the ITI period were removed from the analysis, as well as neurons from incomplete sessions. Neurons were then further filtered based on whether they were visually responsive or not. For our delay study (Chapter 3), we conducted paired t-tests with Keppel's (1982) modified Bonferroni correction ($p < 0.033$) between the average activity in the middle 5 s of the ITI of each trial type and the average activity from the 300 ms sample period, separately for both stimuli. For our categorisation studies (Chapters 4-7) a neuron was determined to be visually responsive by using a two-way repeated-measures ANOVA with period (the middle 5 s of the ITI and a 300 ms stimulus period) and stimuli (Category 1 vs Category 2) as factors, with repeated measures over stimuli (Greenhouse-Geisser corrected, and Keppel's modified Bonferroni correction, $p < 0.05$; slight exceptions to this method are outlined in Chapters 5 and 6). If a neuron showed a significant difference in firing rate between the ITI and stimulus period for at least one of the stimuli (i.e. a main effect of

period), then the neuron was labelled as being visually responsive. In both cases, the modified Bonferroni correction was calculated on the basis of the number of comparisons used during the visual responsiveness analysis (Keppel, 1982).

Neurons were further classified as being either "excitatory" if activity during the sample period increased significantly from baseline ITI activity, or "inhibitory" if sample activity decreased significantly from baseline ITI activity. Note that in this case, the terms "excitatory" and "inhibitory" do not refer to classic histological meanings, but rather reflect whether the change in activity is greater (excitatory) or less (inhibitory) than baseline activity, a convention we have followed in all our previous papers (Johnston, Anderson, & Colombo, 2017a; 2017b).

Each trial within a session was divided into 50 ms bins. Data was then normalised by the maximum value in the 5 s ITI for each neuron. Because there is no visual stimulation nor any behavioural requirements during the ITI period, we consider ITI activity to represent baseline activity and thus normalised all neural data by the maximum value from this period. For each neuron, we then split the sessions' data into either the two different categories (Chapters 4, 6 and 7) or the number of different stimuli (Chapters 3, 5, and 6) and averaged the neural activity within each of these trial types. Thus, average activity to each category or stimulus was calculated from each neuron.

CHAPTER 3 | INVESTIGATING DELAY ACTIVITY IN THE VISUAL WULST

Anderson, C., Johnston, M., Marrs, E. J., Porter, B., & Colombo, M. (2020). Delay activity in the Wulst of pigeons (*Columba livia*) represents correlates of both sample and reward information. *Neurobiology of Learning and Memory*, *171*, 107214.

Author contributions: CA designed and conducted the experiment, analysed the data, and wrote the manuscript. MJ assisted in conducting the experiment and analysing the data. EJM assisted in conducting the experiment and analysing the data. BP wrote the analysis software. MC assisted in designing the experiment, wrote the analysis software, assisted with reviewing and editing the manuscript, and supervised the project.

In this first study, we wanted to investigate the Wulst, the second intermediary visual area in pigeons, as a potential area of interest (in addition to NCL and ENTO) for subsequent categorisation studies. As there have been no single-unit electrophysiological studies examining delay activity in the Wulst prior to this experiment, we decided to examine the Wulst using single-unit electrophysiology while birds performed a simple delayed matching-to-sample (DMS) task, so that neural data from the Wulst could be compared to similar neural data from NCL and ENTO. The majority of this Chapter is taken directly from the publication above, particularly the Methods and Results sections. The

Introduction and Discussion have been amended in order to better integrate the publication into the thesis.

3.1. Introduction

3.1.1 Anatomy and architecture of the Wulst

The second main visual pathway in birds is the *thalamofugal* pathway, which runs from the retina through the nucleus geniculatus pars dorsalis (GLd) and ends in the telencephalon in an area known as the Wulst and is considered to be analogous to the mammalian *geniculostriate* pathway (Shimizu & Karten, 1993). In birds, the Wulst can be divided into four subdivisions: the hyperpallium apicale (HA), the hyperpallium intercalatum (HI), the hyperpallium densocellulare (HD), and the nucleus interstitialis hyperpalli apicalis (IHA; Reiner et al., 2004; see Figure 3-1 for an illustration). The Wulst is considered to be analogous to the primate striate cortex (Karten, Hodos, Nauta, & Revzin, 1973; Pettigrew, 1979), as neurons in both striate cortex and the Wulst have small receptive fields with considerable retinotopic mapping (Allman & Kaas, 1971; Cowey, 1964; Gattass, Gross, & Sandell, 1981; Miceli, Gioanni, Reperant, & Peyrichoux, 1979; Revzin, 1969; Talbot & Marshall, 1941), although neurons in HD have larger receptive fields than the other subdivisions (Gusel'nikov, Morenkov, & Hunh, 1977; for a review, see Clark & Colombo, 2020). Projections from the GLd mainly terminate in the IHA (Karten et al., 1973), which then projects to the other three subdivisions (Atoji, Sarkar, & Wild, 2018; Kröner & Güntürkün, 1999; Shimizu, Cox, & Karten, 1995), as well as to the NCL (Leutgeb, Husband, Riters, Shimizu, & Bingman, 1996). HI and HD project to HA (Shimizu et al., 1995), and all three have minor projections to NCL (Atoji et al., 2018). HA also has topographical projections to the optic tectum, which is part of the tectofugal pathway described in section 1.2.4. (Karten et al., 1973).

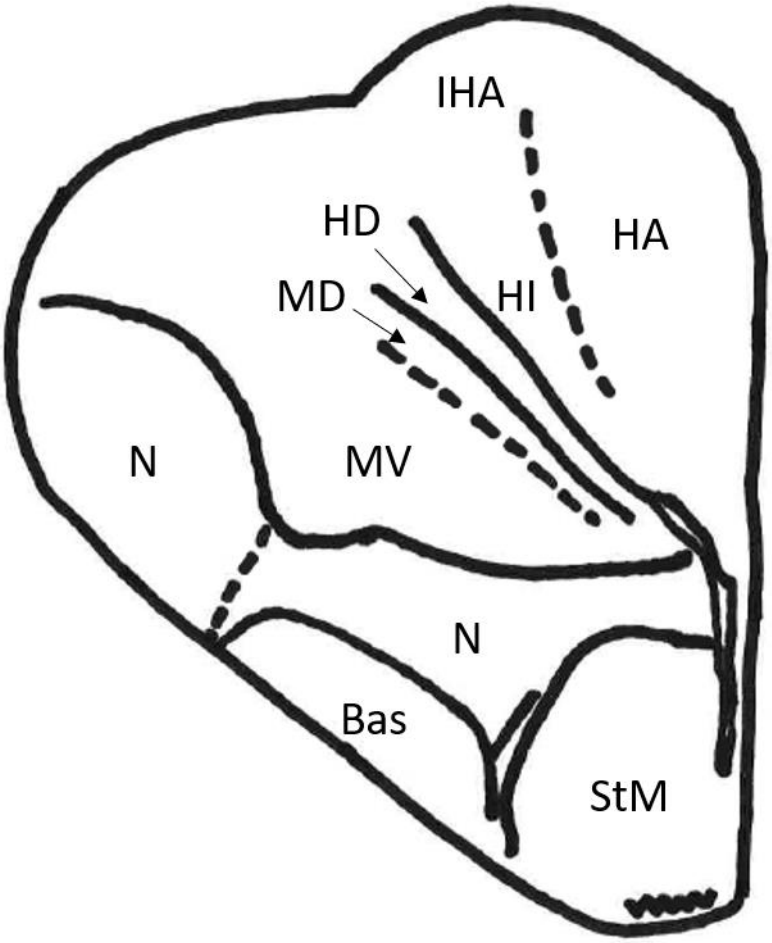

Figure 3-1: Illustration of the Wulst and the surrounding areas.

Brain regions as defined by Reiner et al. (2004): Bas, nucleus basorostralis pallii; HA, hyperpallium apicale; HD, hyperpallium densocellulare; HI, hyperallium intercalatum; IHA, nucleus interstitialis hyperpallii apicalis; MD, mesopallium dorsale; MV, mesopallium ventral; N, nidopallium; StM, striatum mediale.

3.1.2. The function of the Wulst

Paralleling the distinction between striate cortex and IT cortex, where the former causes fewer impairments in pattern discrimination than the latter (Cowey & Weiskrantz, 1967; Gross, 1973), lesions to the Wulst also cause far fewer impairments in pattern discrimination compared to lesions of ENTO (Bessette & Hodos, 1989; Pasternak & Hodos, 1977; Watanabe, 1992). Instead, rather than being mainly involved in pattern and motion processing (the "what" of a stimulus), Watanabe and colleagues (2003; Watanabe, Mayer, & Bischof, 2011) have argued that the Wulst is involved in visuo-spatial learning (the "where" of a stimulus). Similarly, striate cortex in primates also seems to store and encode visuo-spatial information during a delayed-response (DR) task and is thought to be a link between sensory and working memory (Supèr, Spekreijse, & Lamme, 2001).

There is some evidence that the Wulst, like ENTO, may be important for visual memory. Pasternak (1977) lesioned either the Wulst or the surrounding area after pigeons had been trained on a DMS task with varying delays (from 0 s to 8 s) and found that following surgery, the performance of all birds fell to chance levels. Birds with surrounding area damage were able to relearn the task to preoperative levels at all delays. In contrast, birds with Wulst lesions could, with extensive training, relearn the DMS task with a 0 s delay, but were generally unable to relearn the task with delays longer than 0 s. Given the Wulst's potential role in visual working memory, the first goal of the current study was to examine whether neurons in the Wulst display delay activity, that is, activity indicative of a neural correlate of the animal remembering the sample stimulus. Interestingly, Pasternak (1977) noted that Wulst lesions also appeared to cause perseverative biases, in that the birds did not adapt their behaviour as a function of an

incorrect response. Pasternak (1977) therefore argued that Wulst lesions may also cause impairments in integrating reward information to control behaviour.

3.1.3. The delayed matching-to-sample (DMS) task

We have recently explored the influence of reward information on delay activity in a number of pallial areas such as ENTO, as well as the NCL (Johnston et al., 2017a; 2017b). The task that we have used to explore the influence of reward is a modified version of the DMS task. In the standard DMS task, also known as a common-outcomes (CO) DMS task, the animal is rewarded after every correct response, irrespective of which stimulus served as the sample. A slightly modified version of the DMS task is the differential outcomes (DO) DMS task, which follows the same procedure as the CO DMS task, but instead of rewarding subjects after a correct response following either sample stimuli, in the DO task reward is delivered only after correct responses following one of the sample stimuli, and not the other. Across both CO and DO tasks, animals are required to respond to the correct comparison following either sample stimulus, otherwise a time-out punishment is delivered (in the current study's case, signalled by a 500 Hz tone), and the animals are forced to repeat the trial until they respond correctly (correction routine; for a more comprehensive illustration, refer to Figure 3-2). While the two tasks require the same behavioural response from the birds, we believe that encoding may differ between the two tasks on a neuronal level, as one task has differential reward outcomes tied to the stimuli (DO), but the other task does not (CO; for a comprehensive review of the DMS task, see Anderson & Colombo, 2019).

From the neural activity observed during a DO DMS task, it becomes clear that delay activity in NCL is more likely to be related to anticipation of reward, rather than retention of visual information (Johnston et al., 2017a). The reason is that NCL delay activity

occurs mostly after the sample stimulus associated with reward, and not after the sample stimulus not associated with reward, despite the fact that to successfully solve the task the animal must engage working memory following both sample stimuli. In contrast, in ENTO, although the delay activity is modulated by reward in that it is less prominent after the sample that is not associated with reward compared to the sample stimulus that is associated with reward, the key difference to NCL is that delay activity does occur following both sample stimuli. The overall conclusion is that NCL delay activity is more likely a neural correlate of reward anticipation, whereas ENTO delay activity is more likely a neural correlate of working memory for the sample stimulus. In both NCL and ENTO, however, it is likely that both reward and sample information are being coded, but that one type is more dominant than the other.

3.1.4. Aims and objectives of the study

As stated in section 3.1.2., the first goal of the current study was to examine whether neurons in the Wulst display delay activity like we have previously seen in NCL and ENTO; that is, whether Wulst neurons display activity indicative of a neural correlate of the pigeon remembering the sample stimulus. Given the possibility that the Wulst may support some reward processing (Pasternak, 1977), the second goal of the current experiment was to explore Wulst delay activity under both CO and DO conditions. If the Wulst is a structure that predominantly supports visual working memory we would expect to see, like in ENTO, delay activity following both sample stimuli on both the CO and DO DMS tasks. In contrast, if the Wulst is a structure that predominantly supports processing information about the reward, then we would expect delay activity following both sample stimuli during the CO DMS task (as both sample stimuli predict the possibility to obtain a reward), but during the DO DMS task we would expect to see delay

activity restricted to the sample stimulus associated with reward, but not to the sample stimulus not associated with reward.

3.2. Materials and Methods

3.2.1. Subjects

Four experimentally sophisticated pigeons (*Columba livia*) were used as the subjects for the current experiment. All four birds had previously served in a study using a DMS task (Kouwenhoven & Colombo, 2016).

3.2.2. Apparatus and stimuli

For the current study, the Perspex panels that were positioned in front of the monitors had six square holes arranged in a two (rows) x three (columns) grid. Each of the square holes measured 60 x 60 mm and were spaced 65 mm apart from centre to centre. The sample stimuli were displayed in the middle square of the top row, and comparison stimuli were displayed in the two outer squares of the top row. Black-and-white photographs of a skateboarder and a flower served as the two stimuli.

3.2.3. Behavioural task

Each trial began with a 10 s intertrial interval (ITI). Following the ITI, one of the sample stimuli (skateboarder or flower) was presented in the middle square. Birds were required to peck the sample stimulus three times in order to turn off the stimulus and initiate the delay period. After the 3 s delay period, the two comparison stimuli (skateboarder and flower) were presented in the outer two squares. Pigeons were required to peck the comparison stimulus that matched the sample stimulus they had seen before the delay. Two of the birds (M6 and M11) were trained on the CO DMS task, in which a

correct response to either the skateboarder or flower comparison stimulus trials resulted in 2.5 s access to reward (see Figure 3-2a–c). The other two birds (M8 and M10) were trained on the DO DMS task, in which only correct responses to the skateboarder comparison stimulus resulted in reward, while correct responses to the flower comparison stimulus were not rewarded (see Figure 3-2d–f). During correct flower trials the hopper light still illuminated. Incorrect responses to skateboarder and flower trials for both sets of birds were punished with a time-out signalled by a 500 Hz tone (65 dB) for 1 s.

A correction routine was in place for both training and testing, such that all incorrect trials were repeated until the correct response was made. Only the first attempt at each trial was calculated for behavioural performance. Each session consisted of completing 64 correct trials. On average, across these 64 trials, M6 did 8 repeat trials, M11 did 10 repeat trials, M10 did 6 repeat trials, and M8 did 10 repeat trials. For each session, the skateboarder stimulus served as the sample on half of the trials, and the flower stimulus served as the sample on the other half, randomly intermixed. The left-right positions of the comparison stimuli were balanced across the trials, with the skateboarder on the left and flower on the right on half the trials, and vice versa on the other half of the trials, also randomly intermixed.

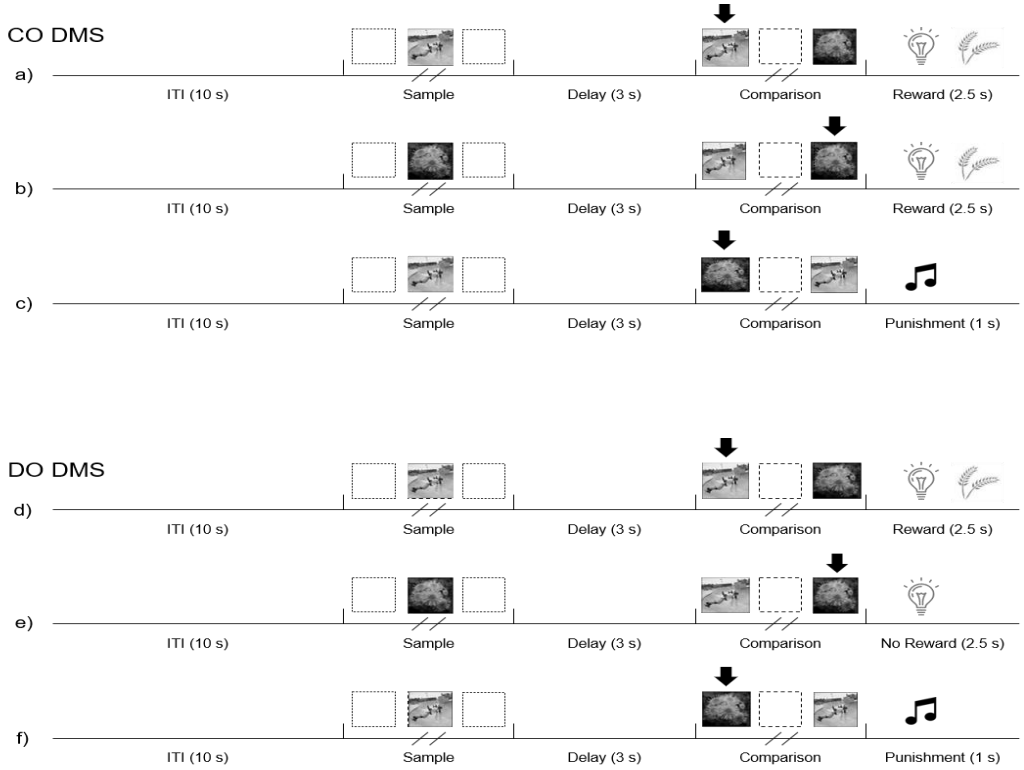

Figure 3-2: Behavioural task.

The sequence of events on the behavioural task for the common outcomes (CO) procedure (a–c) and the differential outcomes (DO) procedure (d–f). For the CO procedure, when the skateboard (a) or the flower (b) served as the sample stimulus, a correct response during the comparison period resulted in a wheat reward. If the wrong comparison stimulus was chosen (c), the birds were not rewarded, and a time-out punishment period ensued. For the DO procedure, when the skateboard served as the sample stimulus (d), a correct response in the comparison period resulted in wheat reward, but if the flower was the sample stimulus (e), a correct response during the comparison period did not yield a reward but did result in the hopper light being illuminated. As in the CO procedure, an incorrect response during the comparison period of the DO procedure (f) resulted in a time-out punishment period.

3.2.4. Training protocol

In a previous study, the pigeons were magazine trained to eat from the hopper, and then autoshaped to peck a white dot stimulus. Once pigeons were reliably pecking the dot, they were trained to peck the skateboarder and flower stimuli used in the current experiment. Following reliable pecking to these two stimuli, the birds were trained on the DMS task, starting with no delay. An 80% accuracy criterion was used to advance birds through delay periods of 0 s (no delay), 0.5 s, 1 s, and finally 3 s. The birds underwent surgery once they were performing at 80% correct for both skateboarder and flower trials with a 3 s delay.

3.2.5. Surgery

All four birds had microdrives installed in the Wulst region at AP +11.0 and ML ±3.0 (Karten & Hodos, 1967). For the two birds trained on the CO procedure, one had a microdrive inserted in the left hemisphere (M6), and the other in the right hemisphere (M11). For the two birds trained on the DO procedure, one had a microdrive inserted in the left hemisphere (M8), and the other in the right hemisphere (M10).

3.2.6. Neural analysis

To measure whether a neuron fired to the sample stimulus, we analysed the activity during a 300 ms period between –400 ms to –100 ms prior to the first of the three required pecks to the sample stimulus (‘stimulus last’ period). For the comparison period, we measured the same 300 ms period before the only peck required. For the delay period, we measured the activity across the entire 3 s delay separately for skateboarder and flower trials.

3.3. Results

3.3.1. Histology

All electrode tracks were within the targeted Wulst region as defined by Karten and Hodos (1967). Figure 3-3 shows the reconstructed tracks for three of the four birds. The intended track positions were AP +11.0 and ML ±3.0. The track position for the left hemisphere CO bird (M6; solid red line) was AP +11.5 and ML +3.5, differing from both the intended AP and ML positions by 0.5 mm. The track position for the right hemisphere CO bird (M11; solid blue line) was AP +11.0 and ML –3.2, differing only from the intended ML position by 0.2 mm. The track position for the left hemisphere DO bird (M8; red star) could not be recovered, although the termination point was recovered at AP +10.5 and ML +3.0, differing only from the intended AP position by 0.5 mm. The track position for the right hemisphere DO bird (M10; dashed blue line) was AP +11.5 and ML –4.2, differing from the intended AP position by 0.5 mm and the intended ML position by 1.2 mm.

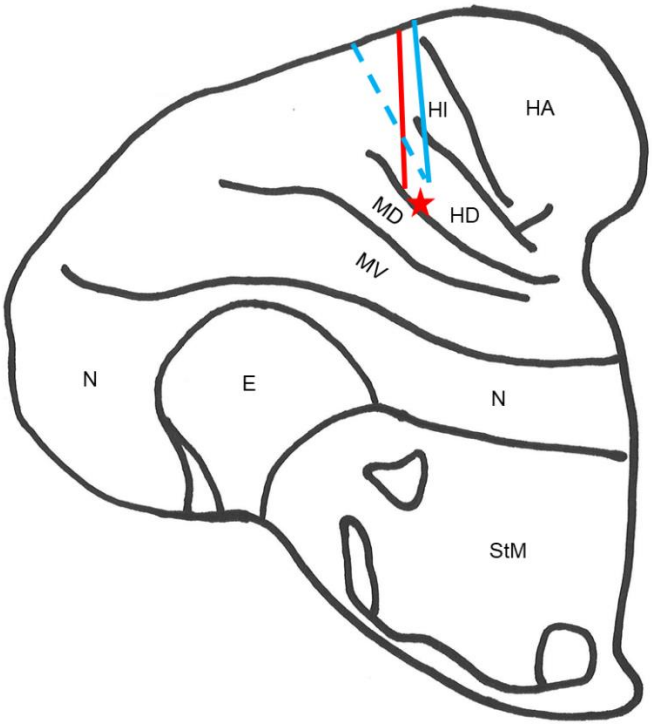

Figure 3-2: Electrode track reconstruction.

The solid blue line is the right hemisphere CO bird (M11) and the solid red line is the left hemisphere CO bird (M6). The dashed blue line is the right hemisphere DO bird (M10). Unfortunately, the track could not be recovered for the left hemisphere DO bird (M8), although the termination point was recovered, and is indicated by the red star. Brain regions (as defined by Reiner et al., 2004): E, entopallium; HA, hyperpallium apicale; HD, hyperpallium densocellulare; HI, hyperpallium intercalatum; MD, mesopallium dorsale; MV, mesopallium ventrale; N, nidopallium; StM, striatum mediale.

3.3.2. Behavioural performance

The behavioural performance of both the CO and DO birds on skateboarder and flower trials is shown in Figure 3-4. The average performance of all birds was analysed using a two-way repeated-measures analysis of variance (ANOVA) with Task Type (2: CO vs. DO) and Stimulus (2: skateboarder vs. flower) as factors, with repeated-measures over Stimulus (Greenhouse-Geisser corrected). There was a significant main effect of

Stimulus, $F(1, 58) = 69.18$, $p < 0.001$, and Task Type, $F(1, 58) = 15.23$, $p < 0.001$, and a significant interaction effect between Task Type and Stimulus, $F(1, 58) = 64.83$, $p < 0.001$. Paired t-tests showed that the main effect of Stimulus was only significant for DO birds, $t(58) = 10.11$, $p < 0.001$, and not significant for CO birds, $t(58) = 0.42$, $p = 0.67$. The fact that birds trained on the DO DMS task perform more accurately during rewarded trials than unrewarded trials, but that this difference in performance is not seen on the CO DMS task, is in line with previous findings (Johnston et al., 2017a; 2017b). Overall, birds in the DO condition performed more accurately than birds in the CO condition, which is also in line with Johnston et al. (2017a; 2017b). Regardless of the differences in performance between DO and CO trained birds, all four birds performed significantly above chance during both skateboarder, $t(3) = 26.43$, $p < 0.001$ and flower trials, $t(3) = 40.73$, $p < 0.001$.

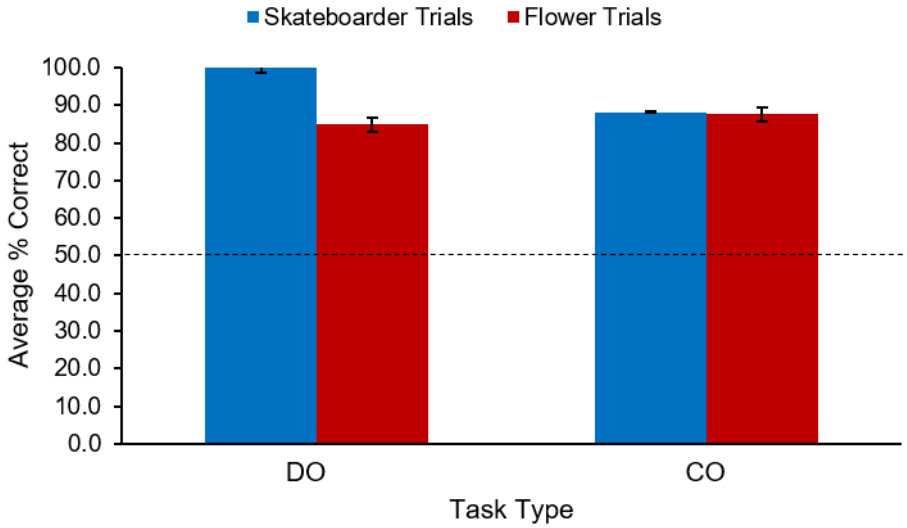

Figure 3-3: Overall behavioural performance on the CO DMS and DO DMS tasks.

The percent correct on skateboarder and flower trials is averaged across all sessions of the two DO birds (M8 and M10), and the two CO birds (M6 and M11). The dashed line denotes chance performance (50%), and error bars are ±1 SEM. DO, differential outcomes; CO, common outcomes.

3.3.3. Visually responsive neurons

Overall, we recorded activity from 50 neurons from the Wulst of the two CO birds, and 59 neurons from the Wulst of the two DO birds. A full breakdown of the visually responsive neurons found in CO and DO birds is shown in Table 3-1. Neurons were then further classed as being selective or non-selective. Selective visual neurons were neurons that either fired to one stimulus only (en/ne/in/ni), or fired to both stimuli but in different manners, i.e. excitatory to one stimulus and inhibitory to the other (ie/ei), or excitatory/inhibitory to both but to different extents (e>e/e<e,i>i,i<i). Non-selective visual neurons were neurons that fired to both stimuli to an equal extent (e=e/i=i).

Table 3-1

Breakdown of Visually Non-Selective and Selective Neurons Across Both CO and DO Tasks

	Non-Selective Neurons			Selective Neurons										
	e=e	i=i	Total	e>e	e<e	i>i	i<i	ei	ie	en	ne	in	ni	Total
CO	7 (77.8%)	2 (22.2%)	9	0 (0.0%)	3 (17.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (64.7%)	3 (17.6%)	0 (0.0%)	17
DO	1 (20.0%)	4 (80.0%)	5	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (9.1%)	1 (3.0%)	0 (0.0%)	12 (36.4%)	1 (3.0%)	7 (21.2%)	9 (27.3%)	33

Note: e = excitatory; i = inhibitory; n = not significant from baseline. The first letter in each pair refers to the type of activity on skateboarder trials, and the second letter refers to the type of activity on flower trials.

Of the 50 neurons recorded from CO birds, 26 (52.0%) were found to be visually responsive. Of these 26 visually responsive neurons, 9 (34.6%) were non-selective while the remaining 17 (65.4%) were stimulus selective. Of the 59 neurons recorded from the DO birds, 38 (64.4%) were visually responsive. Of these 38 visually responsive neurons, 5 (13.2%) were non-selective while the remaining 33 visually responsive neurons (86.8%) were stimulus selective. The vast majority of the selective neurons collected during the CO and DO tasks were of the en, ne, in, and ni variety.

As the Wulst is comprised of four subdivisions (HA, IHA, HI, and HD), we divided the neurons recorded from each bird into those within the two subdivisions that were within our electrode tracks (HI and HD) to see if there was any difference in the visual coding properties between subdivisions. Across all four birds, the number of visually responsive cells was exactly the same between the two subdivisions (32 neurons in HI and 32 in HD), as well as the numbers of selective and non-selective neurons in these subdivisions (7 non-selective and 25 selective in HI; 7 non-selective and 25 selective in HI). The only pattern of difference that we could find between the two subdivisions was that in HI, more neurons tended to be inhibitory, while more neurons tended to be excitatory in HD (although both types were present in both areas). Overall, there seems to be no difference between these subdivisions and their visual coding properties.

3.3.4. Delay neurons

A full breakdown of the different types of delay neurons found in CO and DO birds is shown in Table 3-2. As with visually responsive neurons, we labelled neurons as delay neurons if activity during the delay period was significantly different from baseline ITI activity. To determine whether neurons were delay neurons, we used paired t-tests with Keppel's (1982) modified Bonferroni correction ($p < 0.033$) between the average activity

of the 3 s delay period and the average activity of the middle 5 s of the ITI, for both skateboarder and flower trials separately. Neurons were then further classified as being either excitatory or inhibitory. Again, as with visually responsive neurons, delay neurons were then further classed as being selective or non-selective. Selective delay neurons were neurons that either fired to one stimulus only (en/ne/in/ni), or fired to both stimuli but in different manners, i.e. excitatory to one stimulus and inhibitory to the other (ie/ei), or excitatory/inhibitory to both but to different extents (e>e/e<e,i>i,i<i). Non-selective delay neurons were neurons which fired to both stimuli to an equal extent (e=e/i=i).

Table 3-2

Breakdown of Selective and Non-Selective Delay Neurons Across Both CO and DO Tasks

	Non-Selective Neurons			Selective Neurons										
	e=e	i=i	Total	e>e	e<e	i>i	i<i	ei	ie	en	ne	in	ni	Total
CO	12 (66.7%)	6 (33.3%)	18	1 (7.1%)	0 (0.0%)	0 (0.0%)	2 (14.3%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	5 (35.7%)	4 (28.6%)	1 (7.1%)	14
DO	0 (0.0%)	3 (100.0%)	3	1 (2.9%)	0 (0.0%)	1 (2.9%)	2 (5.7%)	1 (2.9%)	1 (2.9%)	12 (34.3%)	6 (17.1%)	8 (22.9%)	3 (8.6%)	35

Note: e = excitatory; i = inhibitory; n = not significant from baseline. The first letter in each pair refers to the type of activity on skateboarder trials, and the second letter refers to the type of activity on flower trials.

For the 50 neurons recorded from the CO birds, 32 (64.0%) of these neurons were found to be delay neurons. Of the 32 CO delay neurons, 13 (40.6%) neurons fired in an excitatory manner during the delay period of both skateboarder and flower trials, with the majority of those neurons (12/13) being non-selective, and one showing greater excitatory activity during skateboarder trials over flower trials. (Table 3-2, ee; see also Figure 3-5a for an example). 8 (25.0%) of the neurons fired in an inhibitory manner during the delay period of both skateboarder and flower trials, with 6/8 being non-selective, and 2/8 showing greater inhibitory activity during skateboarder trials over flower trials (Table 3-2, ii; see also Figure 3-5b for an example).

For the 59 neurons recorded from DO birds, 38 (64.4%) of these neurons were found to be delay neurons. Of the 38 DO delay neurons, 12 (31.6%) were excitatory during skateboarder trials exclusively (Table 3-2, en; see also Figure 3-5c for an example). 8 (21.1%) of the neurons similarly were inhibitory during skateboarder trials exclusively (Table 3-2, in; see also Figure 3-5d for an example). Furthermore, 1 (2.6%) neuron showed selectivity for skateboarder trials over flower trials despite being excitatory during both trial types (e>e), and 2 (5.3%) neurons showed selectivity for skateboarder trials over flower trials despite being inhibitory to both trial types (i<i).

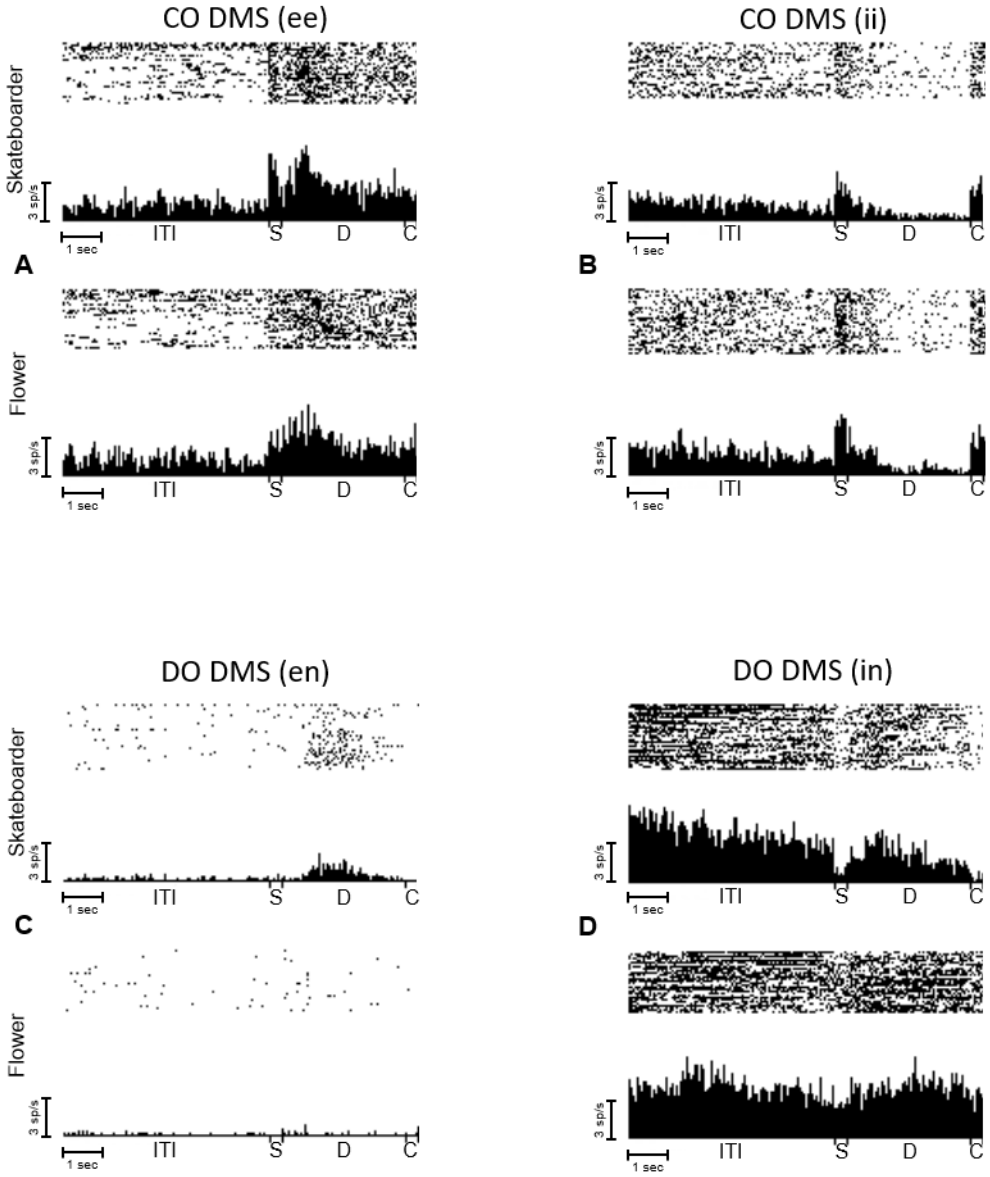

Figure 3-4: Example delay neurons.

A: Neural activity that was excitatory during the delay period during both skateboarder and flower trials in a CO bird. **B:** Neural activity that was inhibitory during the delay period of both skateboarder and flower trials in a CO bird. **C:** Neural activity that was excitatory during the delay period of skateboarder trials but not during flower trials in a DO bird. **D:** Neural activity that was inhibitory during the delay period of skateboarder trials but not flower trials in a DO bird. ITI, intertrial interval; S, sample period; C, comparison period; D, delay period; sp/s, spikes per second. The ITI analysis period was 5000 ms from -7500 to -2500 ms from the onset of the sample stimulus, the sample analysis period was 300 ms from -400 to -100 ms from the first peck to the sample stimulus, the delay analysis period was the full 3000 ms delay period from -3000 ms to onset of the comparison period, and the comparison period was 300 ms from -400 to -100 ms from the peck to the comparison stimulus.

3.3.5. Responsivity across sample and delay periods

We also looked at how the responsivity of each neuron changes between the sample and delay periods. Eight CO neurons (16%) responded in the same way during both the sample and delay – five were excitatory to both stimuli in both periods, two were excitatory during skateboarder trials and not responsive during flower trials, and one was inhibitory to both stimuli during both periods. A further four CO neurons (8%) changed their responding from the sample to the delay period. Of these neurons, three were only excitatory to the flower stimulus during the sample period, but during the delay period activity on flower trials returned to baseline levels, and activity during skateboarder trials became inhibitory. Finally, one neuron was excitatory to both stimuli during the sample period but switched to being inhibitory to both stimuli during the delay period.

Among DO neurons, seven neurons (11.7%) responded in the same way across both sample and delay periods – five neurons were only excitatory during skateboarder trials during both periods, one neuron was excitatory to both stimuli, and one neuron was only inhibitory during flower trials. A further 11 DO neurons (18.6%) changed their responding between the sample and delay periods. Of these 11 neurons, two were only inhibitory to the flower stimulus during the sample but switched to only being inhibitory to the skateboarder stimulus during the delay. Two neurons were only inhibitory to the flower stimulus during the sample but became only excitatory to the skateboarder during the delay. One neuron was only excitatory to the skateboarder stimulus during the sample but became only excitatory on flower trials during the delay. One neuron replicated this same pattern, but with inhibitory activity, while another neuron also switched from inhibitory activity to the skateboarder during the sample, to excitatory activity during flowers trials during the delay. One neuron switched from inhibitory activity to the flower

stimulus in the sample period to excitatory activity during the delay of flower trials, while another neuron went from being excitatory to the skateboarder and inhibitory to the flower, to inhibitory only during the delay of skateboarder trials. Finally, one neuron switched from being inhibitory to both stimuli to excitatory only during skateboarder trials during the delay, and one neuron followed this pattern inversely, going from excitatory only to the skateboarder to inhibitory during the delay of both trial types.

3.3.6. Population profiles

We created four population profiles (CO excitatory, CO inhibitory, DO excitatory, and DO inhibitory) to examine how neurons responded during both skateboarder and flower trials across the ITI, sample, delay, and comparison periods. Each neuron could contribute up to two instances of delay activity (for each sample stimulus), and it could do so in either an excitatory or inhibitory manner. For CO neurons, response profiles included neurons that were either excitatory to at least one of the two stimuli (ee, ei, en, ie, and ne neurons) or inhibitory to at least one of the two stimuli (ii, ie, in, ei, and ni neurons). That is, neurons whose activity was excitatory towards the skateboarder stimulus were compared against neurons whose activity was excitatory towards the flower stimulus, and the same with inhibitory activity. Therefore, for CO neurons, a neuron could contribute either only one or two instances of activity. For DO neurons, response profiles were created based on whether activity on rewarded skateboarder trials was excitatory or inhibitory, regardless of activity on unrewarded flower trials (ee, ei, and en neurons, and ii, ie, and in neurons, respectively). That is, if a neuron responded to the skateboarder stimulus, the same neuron's activity towards the flower stimulus was also used in the response profile. Therefore, for DO neurons, each neuron contributed two instances of activity. Our method of selecting neurons for both CO and DO response profiles are based

on previous conventions (see Johnston et al., 2017a; 2017b), and it should be noted that this preselection of neurons does not affect the patterns of activity that emerge. Neuronal activity from each neuron that contributed to the population profiles was normalised by taking the maximum value of the 100 50 ms ITI bins, and then dividing each of the 50 ms bins (100 ITI bins, 6 sample period bins, 60 delay period bins, and 6 comparison period bins) by that maximum value. We then analysed the population profiles by using four separate two-way repeated-measures ANOVAs with Bin (99, 6, 60, and 6 50 ms bins for the ITI, sample, delay, and comparison periods, respectively) and Stimulus (2: skateboarder vs. flower) as factors, with repeated measures over both Bin and Stimulus (Greenhouse-Geisser corrected).

The CO excitatory profile is shown in Figure 3-6a. Across all analysed periods (ITI, sample, delay, and comparison), there was no significant main effect of Bin, all Fs < 1.63, all ps > 0.16, or a significant main effect of Stimulus, all Fs < 1.54, all ps > 0.24. Furthermore, across all analysed periods, there was no significant interaction between Bin and Stimulus, all Fs < 1.78, all ps > 0.16. Despite appearing as if there is a significant difference between flower and skateboarder trials during both the sample and comparison periods, the results are not significant, likely caused by large variation between each contributing neuron.

The CO inhibitory profile is shown in Figure 3-6b. Across all analysed periods (ITI, sample, delay, and comparison) there was no significant main effect of Bin, all Fs < 1.59, all ps > 0.23, or a significant main effect of Stimulus, all Fs < 1.06, all ps > 0.33. Furthermore, across all analysed periods, there was no significant interaction between Stimulus and Bin, all Fs < 1.34, all ps > 0.29.

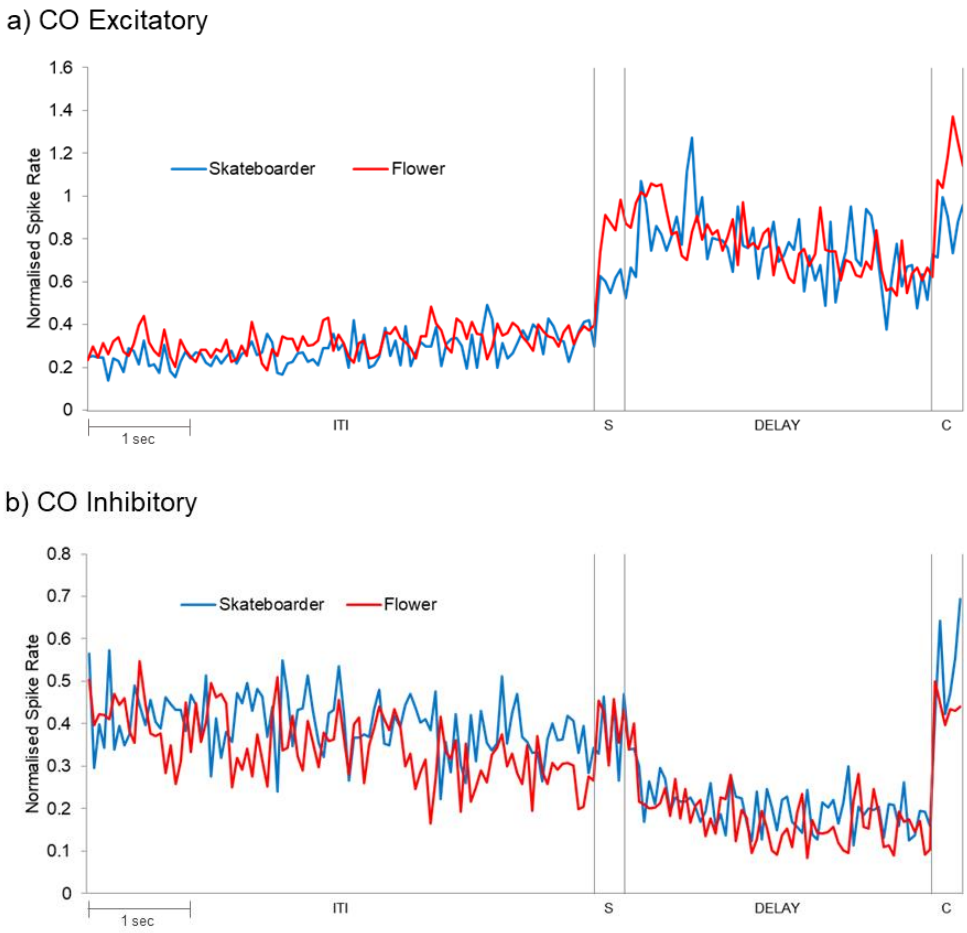

Figure 3-5: CO population profiles.

The population profiles of the (a) 19 excitatory CO neurons and (b) 13 inhibitory CO neurons during skateboarder and flower trials. Of the 19 excitatory neurons, 13 responded to both stimuli (12 e=e and 1 e>e), five responded only to the flower stimulus (ne), and one responded only to the skateboarder stimulus (en). Of the 13 inhibitory neurons, 8 responded to both stimuli (6 i=i and 2 i<i), four responded only to the skateboarder stimulus (in), and one responded only to the flower stimulus (ni). Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. ITI, intertrial interval; S, sample period; C, comparison period. For details on the timings of each period see Figure 3-5.

The DO excitatory profile is shown in Figure 3-7a. Across all analysed periods (ITI, sample, delay, and comparison), there was no significant main effect of Bin, all *F*s < 0.93,

all *p*s > 0.51. There was also no significant main effect of Stimulus during the ITI, $F(1, 13) = 1.43$, $p = 0.25$. However, there was a significant main effect of Stimulus during the sample period, $F(1, 13) = 9.74$, $p = 0.008$, the delay period, $F(1, 13) = 27.42$, $p < 0.001$, and the comparison period, $F(1, 13) = 5.43$, $p = 0.037$. As can be seen in Figure 2.6a, during these three periods, firing during skateboarder (rewarded) trials was significantly more excitatory than firing during flower (unrewarded) trials. There was no significant interaction between Stimulus and Bin across all four periods, all *F*s < 1.01, all *p*s > 0.44.

To see whether delay activity on flower trials remained at baseline ITI levels during the delay period, we used a paired t-test to compare the average ITI firing with the average delay firing. We found that delay firing during unrewarded flower trials was not significantly different from baseline activity, $t(13) = 0.49$, $p = 0.63$. Thus, for DO excitatory neurons, delay activity only occurred during the delay period of rewarded skateboarder trials.

The DO inhibitory profile is shown in Figure 3-7b. Across all analysed periods (ITI, sample, delay, and comparison), there was no significant main effect of Bin, all *F*s < 1.53, all *p*s > 0.14. There was also no significant main effect of Stimulus in the ITI, sample, and comparison periods, all *F*s < 3.02, all *p*s > 0.10. However, during the delay period, there was a significant main effect of Stimulus, $F(1, 14) = 7.90$, $p = 0.014$. As seen in Figure 2.6b, firing during the delay period was more inhibitory during rewarded skateboarder trials compared to the delay period of unrewarded flower trials. There was no significant interaction between Stimulus and Bin, across all four periods, all *F*s < 1.33, all *p*s > 0.27.

To see whether delay activity on flower trials was different to baseline ITI activity, we used a paired t-test to compare the average ITI firing with the average delay firing. We found that delay activity during flower trials was significantly different from baseline activity, $t(14) = 3.90$, $p = 0.002$, indicating that for both trial types delay activity deviated from baseline activity, but to more of an extent during rewarded skateboarder trials than during unrewarded flower trials.

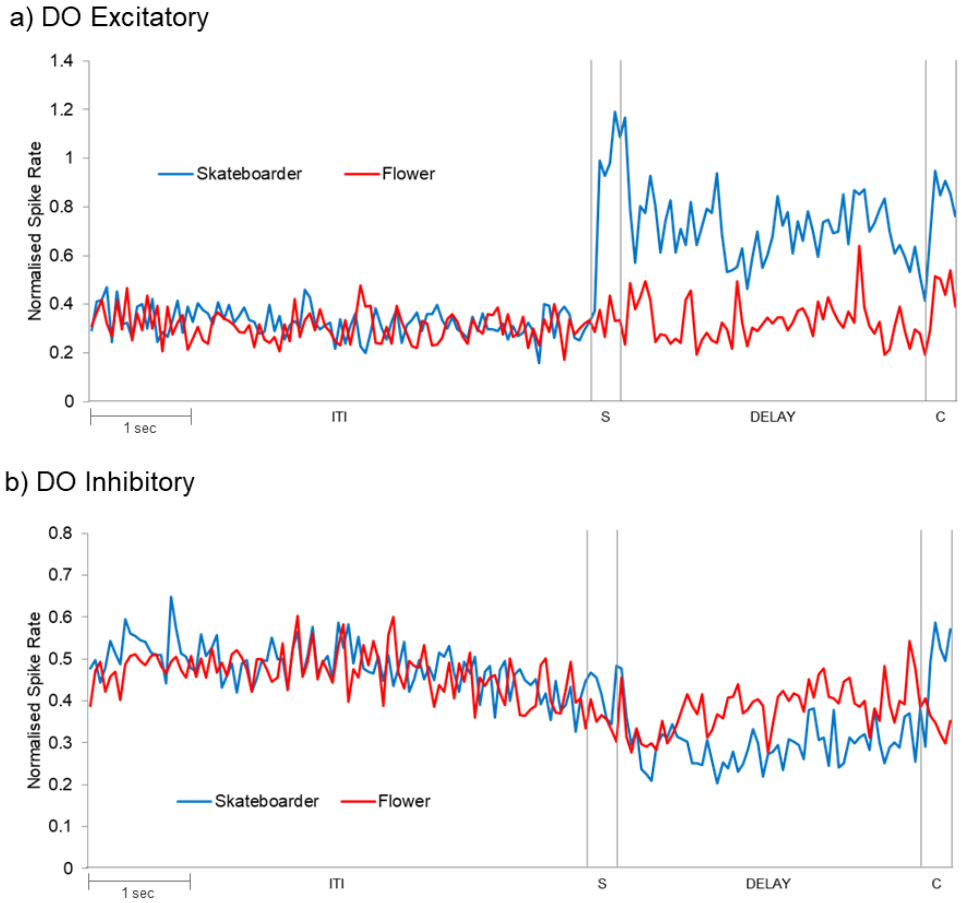

Figure 3-6: DO population profiles.

The population profiles of the (a) 14 excitatory DO neurons and (b) 15 inhibitory DO neurons during skateboarder and flower trials. Of the 14 excitatory neurons, two responded to both stimuli (1 e>e and 1 ei) and 12 responded only to the skateboarder stimulus. Of the 15 inhibitory neurons, 7 responded to both stimuli (3 i=i, 1 i>i, 2 i<i, and 1 ie) and 8 responded only to the skateboarder stimulus (in). Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. ITI, intertrial interval; S, sample period; C, comparison period. For details on the timings of each period see Figure 3-5.

3.4. Discussion

3.4.1. Summary of findings

Thirty-two of fifty (64.0%) neurons in CO birds were classified as delay neurons, that is, activity during the delay period of either skateboarder or flower trials was significantly different from baseline ITI activity. These neurons were then further classified as either excitatory delay neurons (activity during the delay period of at least one stimulus type was excitatory) or inhibitory neurons (activity during the delay period of at least one stimulus type was inhibitory). For CO excitatory neurons, activity during the delay period was significantly different from baseline ITI activity during both skateboarder and flower trials, and there was no significant difference between the level of activity following either trial type. Similarly, CO inhibitory delay neurons also showed significant deviation from baseline activity during both skateboarder and flower trials, again with no significant difference between the trial types.

Thirty-eight of fifty-nine neurons (64.4%) in DO birds were classified as delay neurons, that is, activity during the delay period of skateboarder trials that was significantly different from baseline ITI activity. These neurons were then further classified as either excitatory delay neurons (activity during the delay period of rewarded skateboarder trials was excitatory, regardless of activity on flower trials) or inhibitory delay neurons (activity during the delay period of rewarded skateboarder trials was inhibitory, regardless of activity on flower trials). For neurons that were excitatory during the delay period of skateboarder trials, delay activity on rewarded skateboarder trials was significantly different from activity on unrewarded flower trials. For those same neurons, delay activity never deviated from baseline activity during unrewarded flower trials. For neurons that were inhibitory during the delay period of skateboarder trials, delay activity

on rewarded skateboarder trials was significantly different from activity during unrewarded flower trials, but delay activity during both skateboarder and flower trials was significantly different from baseline activity.

3.4.2. CO delay activity – evidence for a sample code?

For birds who learned the CO DMS task, both excitatory and inhibitory Wulst delay neurons displayed activity that was significantly different from baseline ITI activity, during both skateboarder and flower trials. Therefore, under CO conditions, Wulst neurons appear to be coding for remembering the sample stimulus, and so it is likely that the delay activity represents a neural correlate of working memory for the sample stimulus (what we refer to as sample coding). However, it is possible that under CO conditions Wulst neurons could also be coding reward-based information, as reward contingencies are the same for both stimuli, and activity deviates from baseline in a similar way during both trial types. For 14/32 (43.8%) delay neurons, we can conclusively say that sample coding is being used, as these neurons are selective to one stimulus over the other, even though there is no difference in reward contingencies. Being a visual area, we would expect that the remaining non-selective Wulst neurons are using sample coding over reward coding, but as we will discuss in section 3.4.4., this may not be the case.

These findings are in line with previous CO DMS studies in a number of other areas of the avian brain. Colombo et al. (2001) previously demonstrated that ENTO neurons display the same sustained delay activity to both red and green samples during a CO DMS task and argue that delay activity to both samples represents sample coding and working memory. As stated previously, both ENTO and the Wulst are the termination points of their respective visual pathways in the avian brain (Shimizu & Karten, 1993), and thus it

is not surprising that we find similar sustained delay activity during both skateboarder and flower trials of a CO DMS in both of these areas.

We have also previously shown that neurons in the nidopallium frontolaterale (NFL) display significant delay activity to both red and green sample stimuli during a CO DMS task (Johnston et al., 2017b). NFL is higher-order visual area thought to be analogous to some portion of extrastriate cortex (Clark et al., 2019) and receives afferent projections from both the ENTO and the Wulst (Stacho, Ströckens, Xiao, & Güntürkün, 2016). Even non-visual areas such as NCL, which are known to be involved in working memory and executive functioning (Güntürkün, 2005a; 2005b), demonstrate sustained delay activity to both sample stimuli during a CO DMS task (Johnston et al., 2017b). Therefore, under CO conditions, the Wulst appears to be involved in working memory, and delay activity represents a neural correlate of working memory for the sample stimulus.

3.4.3. DO delay activity – reward or sample code?

For birds who learned the DO DMS task, delay activity of inhibitory Wulst neurons was significantly different from baseline ITI activity during both skateboarder and flower trials. Therefore, much like the CO DMS neurons from the Wulst, under DO conditions inhibitory Wulst neurons appear to be coding information about the to-be-remembered sample stimulus, and so it is likely that delay activity represents a neural correlate of working memory for the sample stimulus. We previously found that both excitatory and inhibitory ENTO neurons also display significant delay activity to both stimuli during a DO DMS task (Johnston et al., 2017a), despite only one of the sample stimuli being associated with reward. However, both ENTO neurons in Johnston et al. (2017a) and inhibitory Wulst neurons in the current study also appear to be modulated by reward to some degree. In the current study, we found that there was a significant effect of stimulus

during the delay period for inhibitory Wulst neurons. That is, while delay activity was significantly different from baseline activity during both skateboarder and flower trials, delay activity during rewarded skateboarder trials was also significantly more inhibitory than delay activity during unrewarded flower trials. In fact, in half of neurons which were inhibitory to both stimuli during the delay, these neurons also showed significantly different inhibitory activity between trial types, suggesting that this sample coding may be modulated by reward. We reported a similar finding in ENTO neurons, where inhibitory delay neurons were significantly more inhibitory during rewarded skateboarder trials than unrewarded flower trials, and excitatory ENTO neurons were excitatory during rewarded skateboarder trials and inhibitory during unrewarded flower trials. Therefore, under DO conditions, inhibitory Wulst neurons appear to follow the same patterns as ENTO neurons in that they are primarily coding remembering sample information, but that this information is also modulated by reward information.

On the other hand, the delay activity of excitatory Wulst neurons was significantly different from baseline ITI activity only during the trials in which the rewarded skateboarder stimulus served as the sample. When the sample was the unrewarded flower stimulus, activity during the delay period did not deviate from baseline. Delay activity during the rewarded skateboarder trials but not unrewarded flower trials of the DO DMS task is consistent with patterns of activity seen in NCL. Previously, we found that NCL neurons displayed sustained delay activity during rewarded trials but not unrewarded trials (Johnston et al., 2017a), a finding which has been consistently seen in other DO studies that has recorded from the NCL (Browning et al., 2011; Rose & Colombo, 2005). As the NCL is considered functionally equivalent to the mammalian PFC, it has, like the PFC, been implicated in reward processing (Koenen, Millar, & Colombo, 2013). The fact

that during a DO DMS task, delay activity occurs following the sample associated with reward, and not at all following the sample that is not associated with reward, is in line with the fact that delay activity in the Wulst (definitely in excitatory neurons, and likely in inhibitory neurons) represents a neural correlate of reward.

3.4.4. Reward-related processing in a visual area?

Our findings indicate that the Wulst, under DO conditions, is either strongly implicated in reward (in the case of excitatory neurons) or is modulated by reward to some extent (in the case of inhibitory neurons). While we have previously shown ENTO neurons to be modulated by reward under DO conditions, ENTO neurons appear to primarily code sample information. However, reward-related processing in visual areas is not unprecedented. Stănişor, van der Togt, Pennartz, and Roelfsema (2013) found that activity in V1 neurons in monkeys was strongly influenced by the relative value of stimuli. In Stănişor et al.'s (2013) study, coloured curve stimuli were associated with either a large, small, or no reward. When the difference in reward between two stimuli was large (i.e., large reward vs. no reward), V1 neuronal activity was higher than when the reward difference between two stimuli was small or equal. In rats, V1 activity is also modulated by reward-timing (Shuler & Bear, 2006). The Wulst has been shown to have the closest anatomical resemblance to area V1 in mammals (Ng, Grabska-Barwińska, Güntürkün, & Jancke, 2010), although functionally, V1 is similar to both the Wulst and certain areas of the optic tectum (Li, Xiao, & Wang, 2007), part of the pathway that terminates at ENTO. In our DO DMS study, the relative reward value of the two sample stimuli was large, in that the skateboarder stimulus was associated with reward and the flower stimulus associated with no reward. Thus, it seems likely that, similar to V1 in mammals, the Wulst in birds may also integrate and process reward-related information.

The fact that the Wulst may serve reward functions is supported by evidence that Wulst neurons project to the NCL (Shimizu & Bowers, 1999). The NCL is highly implicated in reward processing, and not only codes whether reward is available, but also the size (Koenen et al., 2013) and value of the reward (Dykes, Klarer, Porter, Rose, & Colombo, 2018).

Under DO conditions, the NCL appears to use reward coding (Browning et al., 2011; Johnston et al., 2017a; Rose & Colombo, 2005). However, under CO conditions, the NCL codes information about the sample (Johnston et al., 2017b). The NCL, therefore, seems capable of supporting both sample coding as well as reward coding, as the situation demands, although it is likely primarily specialised for reward coding. Similarly, in our study, Wulst neurons may be using sample coding under CO conditions, when there is no difference in the stimulus-reward associations, although as noted in previous sections, this pattern of activity could also be interpreted as reward-related information. However, because differences in stimulus-reward associations exist in DO conditions, Wulst neurons appear to be able to code reward information either as strongly as in NCL, as we see in the excitatory neurons, or perhaps more weakly as in ENTO, as indicated by the reward-modulated sample coding in inhibitory neurons. It is not unreasonable to assume that Wulst neurons may employ different types of encoding across the two tasks, as the DO task is heavily tied to reward outcomes and the CO task is not, despite requiring the same behavioural response. For the Wulst, and perhaps other areas of the pigeon brain, it may be that the stimulus-reward associations unique to the DO task allow neurons to engage in reward-based processing more easily than in the CO task when there are no differential reward outcomes. Overall, it is undeniable that the Wulst, while one of the main visual areas in birds, is heavily influenced by reward information.

3.4.5. Subsequent categorisation studies and the Wulst

As stated in section 1.3., the overarching goal of this thesis is to understand how neural activity in areas that use reward/behaviour-driven information and areas that use visual information differs during categorisation. We believe that both NCL and ENTO serve fairly distinct primary functions (reward processing and visual processing, respectively), although as previously discussed, both of these areas are also capable of integrating other types of information at a secondary level. However, as we have shown in the above study, the function of Wulst neurons is more flexible, in that Wulst neurons process more visual information than NCL neurons and more reward information than ENTO neurons. Therefore, it will be more difficult for us to predict and understand what information Wulst neurons will be processing during categorisation. While the fact that the two main intermediary visual areas (ENTO and Wulst) in the pigeon process visual information differently to one another, it is not within the scope of this thesis to draw comparisons between these two areas. We already know from past lesion studies that ENTO appears to be more involved in visual categorisation than the Wulst (Watanabe et al., 2011) and thus ENTO is a key area of interest. For these reasons, we decided that we would not continue to record from Wulst neurons during our subsequent categorisation studies.

CHAPTER 4 | CATEGORISATION OF ENGLISH WORDS AND NONWORDS BY PIGEONS

In this Chapter, the first of four categorisation studies will be presented, in which birds are trained on a word versus nonword discrimination task. Previous categorisation studies with animals often use categories defined by human language, such as cars versus chairs, despite lacking linguistic abilities (see Chapter 1). Therefore, we thought it would be interesting to add to the behavioural study by Scarf, Boy, Reinert, Devine, Güntürkün, and Colombo (2016) which investigated whether pigeons could be trained to learn short English words and discriminate these from nonwords. For this study, we used single-unit electrophysiology to record neural activity in NCL and ENTO during the word/nonword discrimination task.

4.1. Introduction

4.1.1. Language in humans

According to Bosse (2015), there are two types of knowledge that humans acquire when learning to read: grapheme-phoneme associations and orthographic knowledge. The first type of knowledge, grapheme-phoneme associations, is a basic decoding skill we learn as children. For example, children learn that the sound /p/ is associated with the

written letter ‘p’, but that the sound /s/ is associated with both the letter ‘s’ and also the letter ‘c’. Children then apply these grapheme-phoneme associations in order to generate written words (Hulme & Snowling, 2013).

The second type of knowledge, orthographic knowledge, involves understanding letter identities and positions within specific words in an individual’s vocabulary, and then applying this knowledge to novel words (Bosse, 2015). As children become more experienced readers, they rely less on grapheme-phoneme associations and instead use orthographic knowledge, which generates more fluent reading (Bosse, Chaves, Largy, Valdois, 2015). The term ‘orthography’ itself refers to a set of graphemes (i.e. alphabet) and the accompanying rules (i.e. grammar) for writing a specific language (Seifart, 2008). Orthographic knowledge is especially helpful in learning languages with inconsistent orthographies, such as English (Bosse, 2015).

In particular, orthographic knowledge about letter pairings (bigrams) play an important role in word recognition among literate readers (Duñabeitia, Orihuela, & Carreiras, 2014). For example, the word ‘bird’ has three bigrams: ‘bi’, ‘ir’, and ‘rd’. Bigram frequency refers to how often these bigrams appear in an individual’s pre-existing vocabulary (Rice & Robinson, 1975). Novel words which contain bigrams that appear frequently in the pre-existing vocabulary (high bigram frequency) are more easily learned and assimilated into a vocabulary, while novel words that have a low bigram frequency are more difficult to learn (Broadbent & Gregory, 1968; Rumelhart & Siple, 1974). Furthermore, the size of an individual’s vocabulary is important as larger vocabularies increase the likelihood of encountering bigrams found in previously learned words, which elicits faster and more accurate responses than smaller vocabularies (El-Nasan, Veeramachaneni, & Nagy, 2001;

Mohri, Riley, Hindle, Ljolje, & Pereira, 1998). Thus, it seems that bigram frequencies are useful tool when discriminating between real words and nonwords in humans.

4.1.2. Orthographic processing in baboons

Research surrounding the development of reading written language in humans emphasises a need for prior experience with phoneme-grapheme associations before orthographic knowledge is acquired. That is, a spoken form of language is critical in learning to read in humans. However, in contrast to human research, a study by Grainger, Dufau, Montant, Ziegler, and Fagot (2012) demonstrated that non-human primates are able to acquire orthographic knowledge in the absence of a spoken language. Grainger et al. (2012) trained baboons to discriminate four-letter English words from four-letter strings that were not real words (nonwords; see Figure 4-1). Nonwords were always comprised of one vowel and three consonants, and their bigram frequency was minimised, while the bigram frequency of real words was maximised. Exaggerating the differences in bigram frequencies between words and nonwords allowed Grainger et al. (2012) to test if the baboons could learn the statistical properties of words despite having no prior linguistic knowledge.

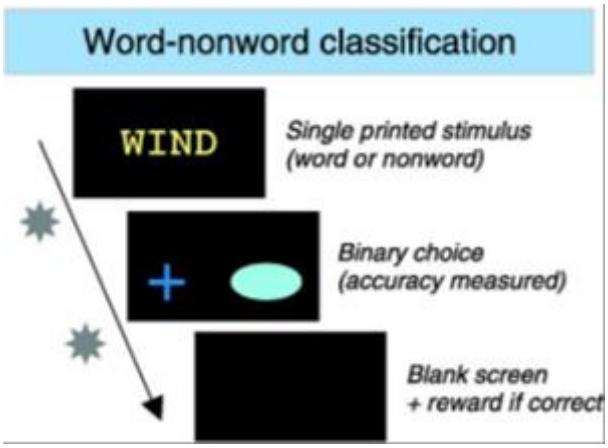

Figure 4-1: The behavioural task from Grainger et al. (2012).

In each session, baboons were presented with a four-letter string which could be a word or a nonword. Baboons were required to choose the oval if the string was a word, and the cross if the string was a nonword. Adapted from Grainger et al. (2012)[4].

At the end of training, baboons had learned between 81 and 308 words. In each session baboons were tested with 25 familiar words they had already learned (representing their pre-existing vocabulary), 25 novel words, and 50 nonwords taken from a pool of over 7,800 nonwords. Baboons were shown one of the four-letter strings (either a word or a nonword), followed by two shapes. The oval represented the 'word' category, while the cross represented the 'nonword' category. Correct categorisation of the strings into words or nonwords resulted in reward. Grainger et al. (2012) found that the higher the bigram frequency of a novel word, the more likely the baboons were able to correctly categorise the string as a word. That is, baboons seem to be sensitive to the statistical properties of words. Similarly, deep convolutional network models that mirror the primate visual area

[4] From Grainger, J., Dufau, S., Montant, M., Ziegler, J. C., & Fagot, J. (2012). Orthographic processing in baboons (Papio papio). Science, 336(6078), 245-248. https://doi.org/10.1126/science.1218152. Reprinted with permission from AAAS and adapted with permission from J. Grainger.

also show that information pertaining to letter positions and pairings, such as bigrams, appears to inform categorisation (Hannagan, Ziegler, Dufau, Fagot, & Grainger, 2014).

4.1.3. Orthographic processing in pigeons

Whether non-primates (and in the case of this thesis, birds) are able to acquire orthographic knowledge has been more widely debated. It has been suggested that pigeons' categorisation and generalisation abilities may mirror the way human children learn words (Wasserman, Brooks, & McMurray, 2015). Indeed, pigeons have been shown to discriminate letters of the alphabet in similar ways to humans, including the types of errors made by both species (Blough, 1982). However, a recent study by Scarf et al. (2016), demonstrated that like baboons, pigeons are also sensitive to the statistical properties of words and can categorise words using this orthographic knowledge. Using the same basic orthographic task as Grainger et al. (2012), Scarf et al. (2016) trained four naïve pigeons to discriminate word and nonword stimuli. Words were drawn from a pool of 308 words learned by the best baboon in Grainger et al. (2012), and nonwords were drawn from the same pool of 7,832 nonwords. The number of words learned by the end of training for each pigeon ranged from 26 to 68 words.

Similar to the procedure run by Grainger et al. (2012), each testing session consisted of 25 familiar words, 25 presentations of the newest word being learned, and 50 nonwords. On each trial, either a word or nonword was presented on-screen, as well as an asterisk symbol above or below the string. The birds were required to peck the four-letter string itself if it was a word, and the asterisk if the string was a nonword (see Figure 4-3 for an illustration of the task). As with the baboons, the pigeons' ability to correctly identify words was correlated with the bigram frequency of the word for both familiar and novel words. Novel words were not only correctly categorised as words more often

than chance, but also were classified as nonwords significantly less often than nonwords were. That is, pigeons' performance when tested with novel words indicated that the pigeons were not simply rote learning their vocabularies.

To further understand whether the pigeons' behaviour resembled hallmark orthographic processing behaviour, the birds were given a transposition test and a substitution test. Transposition probe trials took familiar words and transposed the middle two letters, e.g. 'bird' became 'brid'. In both humans and baboons, transposition of middle letters does not affect the perception of a word, even though the arrangement of letters is no longer a real word (Perea & Lupker, 2004; Ziegler, Hannagan, Dufau, Montant, Fagot, & Grainger, 2013). Substitution probe trials took familiar words and substituted the middle two letters with another random letter from the same category (vowels or consonants), e.g. 'bird' became 'bagd'. Unlike transposed words, substituted words are no longer comprised of the same letter identities as the original word, and both humans and baboons categorise substituted words as nonwords (Duñabeitia et al., 2014; Ziegler et al., 2013). Scarf et al. (2016) found that pigeons performed more similarly to humans on transposition and substitution probes than baboons did, demonstrating that orthographic knowledge and processing is not exclusive to the primate brain (Figure 4-2).

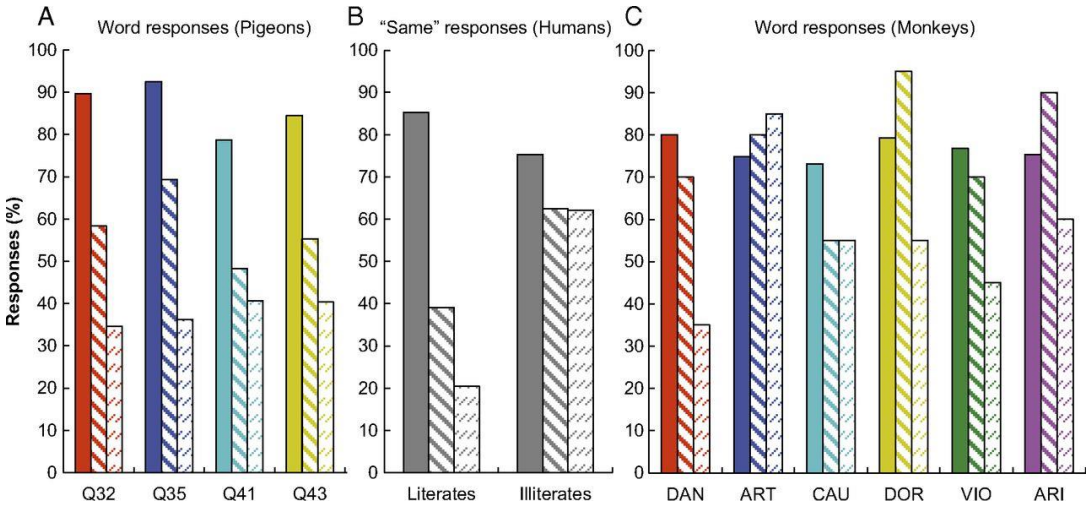

Figure 4-2: Performance on familiar, transposed, and substituted words across species.

The percentage of word responses to familiar words (solid bars), transposed words (diagonal hatch bars), and substituted words (patterned bars) for (a) pigeons (Scarf et al., 2016), (b) humans (Duñabeitia et al., 2014), and (c) baboons (Grainger et al., 2012). From Scarf et al. (2016).[5]

4.1.4. Aims and objectives of the study

The aim of the current study is to further the behavioural evidence presented in Scarf et al. (2016) that pigeons are able to categorise real English words based on their statistical properties with neural evidence from single-unit electrophysiology. As outlined in section 1.3., neural activity from both the NCL and ENTO will be examined, as both areas have been highly implicated in categorisation and categorical processing. In particular, we are interested in how the categories of 'word' and 'nonword' are represented in NCL and ENTO, the involvement of these areas in the categorisation process, and whether differences in categorisation based on bigram frequency are also represented neurally. On

[5] Reprinted with permission from the National Academy of Sciences.

the basis of previous categorisation studies outlined in sections 1.2.3. and 1.2.6., we expect neural activity in NCL to be encoding information based on behavioural outcomes, i.e. reward. On the other hand, we expect that ENTO activity will be encoding categorical information based on differences between words and nonwords, likely characterised by differences in bigram frequency.

4.2. Materials and Methods

4.2.1. Subjects

The subjects were four experimentally sophisticated adult homing pigeons (*Columba livia*). They had previously served in a behavioural study examining orthographic processing in pigeons (Scarf et al., 2016). Unfortunately, our sample size was limited to these four birds as they were the best performing birds from the original study, and the only four to progress their training past the performance recorded in the Scarf et al. (2016) study. Further naïve birds could not be trained due to time limitations, as our four birds had been trained over two years.

4.2.2. Apparatus and stimuli

An operant chamber measuring 360(l) x 440(w) x 470(h) mm internally was used during training and a slightly modified chamber measuring 350(l) x 430(w) x 390(h) mm internally was used during electrophysiological testing. The Perspex panel that was positioned in front of the monitor had one rectangular hole measuring 33(w) x 20(h) mm at the centre of the panel, and was surrounded by four circular holes, each measuring 25 mm in diameter. The right- and left-most circles were placed 50 mm apart from centre to centre, while the top and bottom circles were 28 mm apart, centre to centre. Stimuli were

presented in the rectangular hole and the top and bottom circular holes only. The four-letter strings (which could either be a word or nonword) were presented in the rectangular hole, and the star symbol appeared in the circular holes either above or below the string. The eight-point star symbol measured 15 mm in diameter.

Both words and nonwords were displayed in bolded 12pt Arial font, and all letters were capitalised. Both words and nonwords were taken from the pool of words and nonwords used in Scarf et al.'s (2016) pigeon study (a pool of 308 words and 7,832 nonwords). Nonwords always consisted of three consonants and one vowel, whereas a word could have one or two vowels.

4.2.3. Behavioural task

The birds were trained over three years to learn to discriminate between 30 and 62 words from a pool of 7,800 nonwords (for details, see Scarf et al., 2016). Figure 4-3 shows the sequence of events on word and nonword trials. Trials began with a 5000 ms intertrial interval (ITI) period, during which the birds were presented with a white screen. Following the ITI, a string of four letters, which was either a four-letter word or a nonword, was displayed in the centre of the screen. An asterisk symbol was also presented either above or below the four-letter string. Birds were required to peck the four-letter string if it was a word, or to peck the asterisk symbol if the four-letter string was a nonword. A correct response to both words and nonwords resulted in 2500 ms access to wheat reward. An incorrect response resulted in a punishment consisting of a 5000 ms timeout period, a 500 Hz tone, and no reward. A correction procedure was in place at all times. That is, a trial performed incorrectly was repeated until the pigeon made a correct response. The ITI of the next trial followed either reward delivery or punishment.

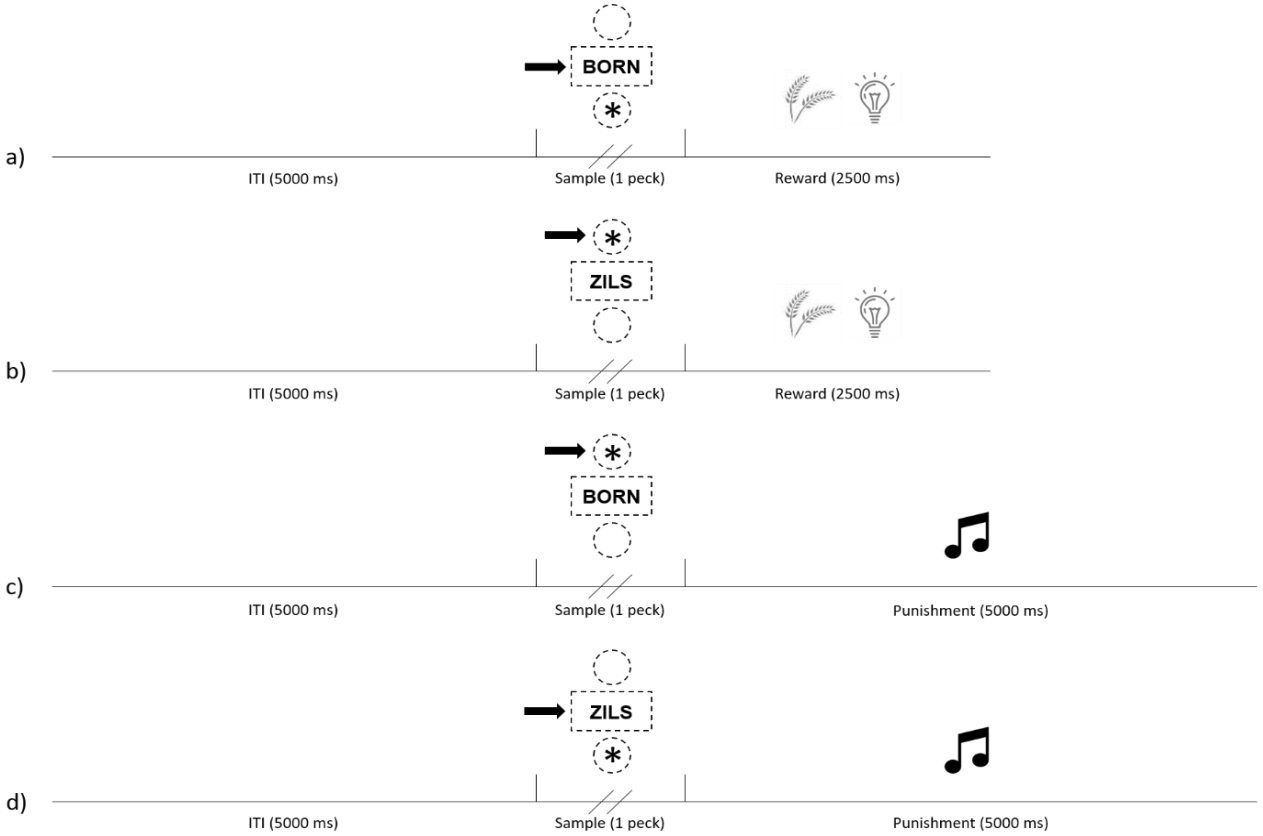

Figure 4-3: Behavioural task.

The sequence of events on word and nonword trials. On word trials, birds were required to peck the string, while on nonword trials they were required to peck the asterisk symbol. When the four-letter string presented in the centre was a real word (a) or a nonword (b), a correct response resulted in 2500 ms access to a food reward (wheat). However, if the birds responded incorrectly to a word (c) or nonword (d), the birds were not rewarded, were given a time-out punishment of 5000 ms signalled by a 500 Hz tone, as had to repeat the trial until they pecked the correct stimulus (correction routine).

In each session, words were displayed for half the trials and nonwords were displayed for the other half, randomly mixed. Each bird had been trained on a different set of words (which we refer to as their 'vocabulary'), which had been chosen from the pool of 308 words from the study by Grainger et al. (2012). All birds had been exposed to the same 7,832 nonwords during their training, and for each testing session a new set of nonwords was pseudo-randomly chosen from the nonword pool such that across the course of training and testing all nonwords were sampled an equal number of times. Q43 had the largest vocabulary (62 words), followed by Q35 (60 words), Q41 (32 words), and Q32 (30 words). The vocabulary of each individual bird is detailed in Figure 4-4. For Q43 and Q35, 62 and 60 nonwords were used in each session, respectively. For Q41 and Q32, each word appeared twice in each session, and 64 and 60 nonwords were used in each session, respectively. Therefore, for Q32 and Q35, each session consisted of 120 trials, while Q43 had 124 trials, and Q41 had 128 trials. The asterisk symbol appeared above the four-letter string on half the trials, and below the string on the other half.

A	B	C	D
BORN	BORN	BORN	BORN
SANG	OMEN	WORK	DONE
ATOM	ACME	GAME	ZOOM
PRON	DOME	GORE	WHAT
SAME	TEAT	CHEM	DAME
LAND	FIAT	PANE	FORE
PITY	CERT	SORT	SAVE
JEER	WELL	WINK	GOAT
LEER	PROM	BOON	LEIS
KRIS	SOME	DOOM	WAND
PAST	MORN	WHIZ	SHIP
TING	MIRE	LINO	LOOK
GROW	BEAK	BEAU	SEEP
MACK	INTO	THEW	THEM
BEAN	EVES	RHEA	HARK
SIRE	PHIS	LEST	WIND
BORE	LOOP	SEAT	WHOM
VERB	HEED	FAME	PROD
DOWN	PURE	BARE	ROOM
CHEM	PARE	HOST	DORY
TIME	HITS	PURE	FOOL
KITE	MANE	WHIP	TINY
TITS	FRET	CHIC	CHID
OVEN	HEAL	HAVE	VEAL
HACK	HERB	DEEM	WHIT
TERM	TORE	MOOD	DOST
YOUR	SEEN	COON	SHAD
WIPE	NAME	CANE	WERT
WORE	BITE	MAST	GREY
LIMY	JUST	BROW	BOAT
	TACK	JERK	CHAR
	DELL	THIN	PREY
		NITS	FRET
		TILL	HARE
		WIGS	LAST
		SACK	MIST
		GORY	LIKE
		WEAL	FORK
		PROW	DOUR
		ZEES	MIEN
		HELL	HALL
		DIRE	MOMS
		WACK	LEEK
		GHEE	NEED
		BAND	SOUL
		BEAN	CITE
		WINS	KITE
		SEEN	HOST
		DOOR	SOON
		DOWN	BANE
		KITE	CERT
		LINK	DOTH
		SURE	TERM
		BANE	LOOT
		ZOOM	GORY
		HANK	PEED
		PRON	BUST
		MANE	DOWN
		MOTH	PURE
		HAND	FAME
			LING
			DIME

Figure 4-4: The vocabularies of each bird.

Q32 learned the smallest number of words (A – 30), followed by Q41 (B- 32), Q35 (C- 60), and Q43 who learned the most words (D- 62).

4.2.4. Surgery

Birds underwent surgery after they had learned their full vocabularies. Two pigeons (Q32 and Q43) had electrodes inserted into NCL, and two other pigeons (Q41 and Q35) had electrodes inserted into ENTO. For both of these brain regions, one microdrive was installed in the left hemisphere in one bird (Q32 in NCL and Q35 in ENTO) and the right hemisphere in the other bird (Q43 in NCL and Q41 in ENTO).

4.3. Results

4.3.1 Histology

All electrode tracks were within the targeted regions as defined by Karten and Hodos (1967). Figure 4-5 shows the reconstructed tracks for the four birds.

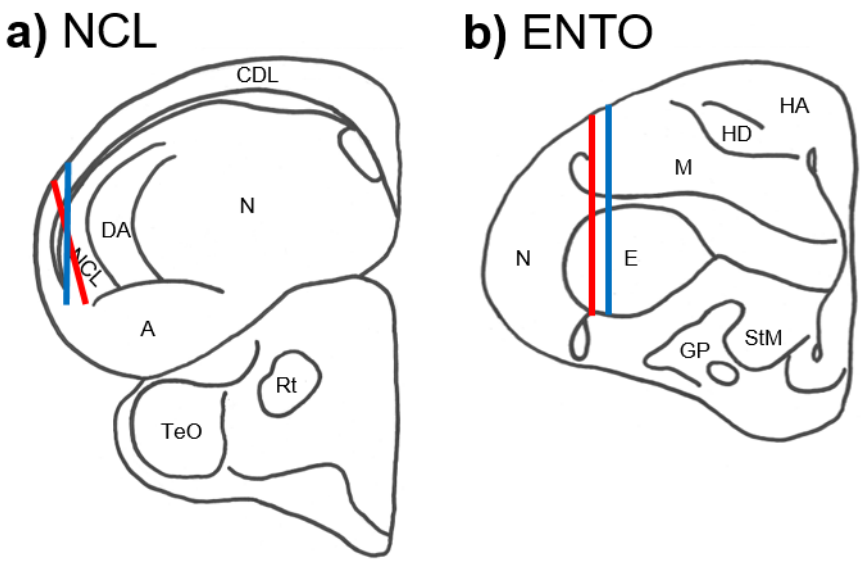

Figure 4-5: The electrode track reconstruction.

(A) NCL. Red line – Q32; blue line – Q43. (B) ENTO. Red line – Q35; blue line – Q41. Brain regions (as defined by Reiner et al., 2004): A, arcopallium; CDL, area corticoidea dorsolateralis; DA, tractus dorso-arcopallialis; E, entopallium; HA, hyperpallium apicale; HD, hyperpallium densocellulare; GP, globus pallidus; M, mesopallium; N, nidopallium; NCL, nidopallium caudolaterale; Rt, nucleus rotundus; StM, striatum mediale; TeO, tectum opticum.

For NCL, the intended track positions were AP +5.5 and ML ±7.5. The track position for the left hemisphere bird (Q32) was AP +4.75 and ML +8.5, differing from the intended AP position by 0.75mm and the ML position by 1.0 mm. The track position for the right hemisphere bird (Q43) was AP +5.75 and ML –8.0, differing from the AP position by 0.25mm and the ML position by 0.5mm.

For ENTO, the intended track positions were AP +9.5 and ML ±6.0. The track position for the left hemisphere bird (Q35) was AP +9.5 and ML +6.5, differing only from the

intended ML position by 0.5 mm. The track position for the right hemisphere bird (Q41) was AP +9.75 and ML –6.0, differing only from the intended AP position by 0.25mm.

4.3.2. Behavioural performance

The behavioural performance across all recording sessions of all four birds, depending on the region they were implanted in, is shown in Figure 4-6. The average performance of all birds was analysed using a 2-way repeated-measures ANOVA with trial type (2: words and nonwords) and brain region (2: NCL and ENTO) as factors, with repeated measures over trial type (Greenhouse-Geisser corrected). There was a significant main effect of trial type, $F(1,157) = 11.29$, $p = 0.001$, and brain region, $F(1,157) = 30.24$, $p < 0.001$, as well as an interaction between trial type and brain region, $F(1,157) = 12.60$, $p = 0.001$. Paired *t*-tests revealed that performance on word trials was significantly different from performance on nonword trials in ENTO, $t(86) = 5.03$, $p < 0.001$, but not in NCL, $t(71) = 0.13$, $p = 0.90$. Regardless of differences in performance for NCL-implanted and ENTO-implanted birds, as well as differences between word and nonword trials in ENTO, performance on all both trial types for both brain regions was significantly above chance, all ts > 20.20, all ps < 0.001.

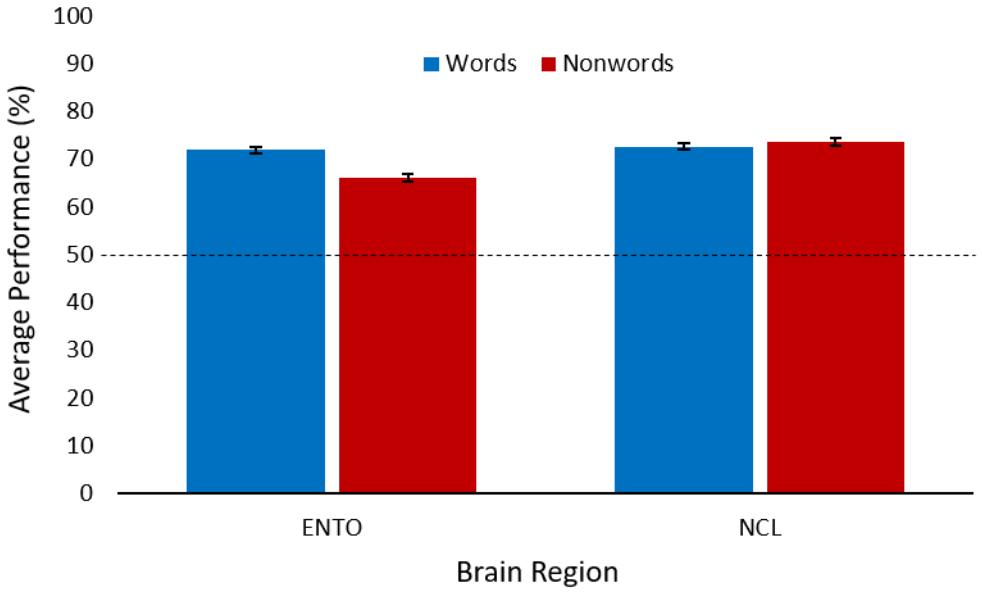

Figure 4-6: Overall behavioural performance on the orthographic task.

The dashed line represents chance levels of performance (50%), and error bars are ±1 SEM.

We also decided to examine whether each bird's behavioural performance on individual words was affected by the bigram frequency of each word, as both Grainger et al. (2012) and Scarf et al. (2016) found that higher bigram frequencies were correlated with better performance. The correlations between behavioural performance for each word and the bigram frequency of the word, across all sessions, for each bird, are shown in Figure 4-7. Note that we have not collapsed data across areas for these correlations, as each bird's vocabulary is unique. For the NCL bird with the small vocabulary (Q32, Figure 4-7a), their average performance for each word was significantly correlated with the bigram frequency of the word, $r = 0.56$, n = 30, $p = 0.01$. For the NCL bird with the large vocabulary (Q43, Figure 4-7b), their average

performance for each word was not significantly correlated with bigram frequency, r = 0.23, n = 62, p = 0.076. For the ENTO bird with the small vocabulary (Q41, Figure 4-7c), their average performance was not significantly correlated with bigram frequency, although it approached significance, r = 0.35, n = 32, p = 0.051. Finally, for the ENTO bird with the large vocabulary (Q35, Figure 4-7d), their average performance on each word was significantly correlated with bigram frequency, r = 0.36, n = 60, p = 0.004.

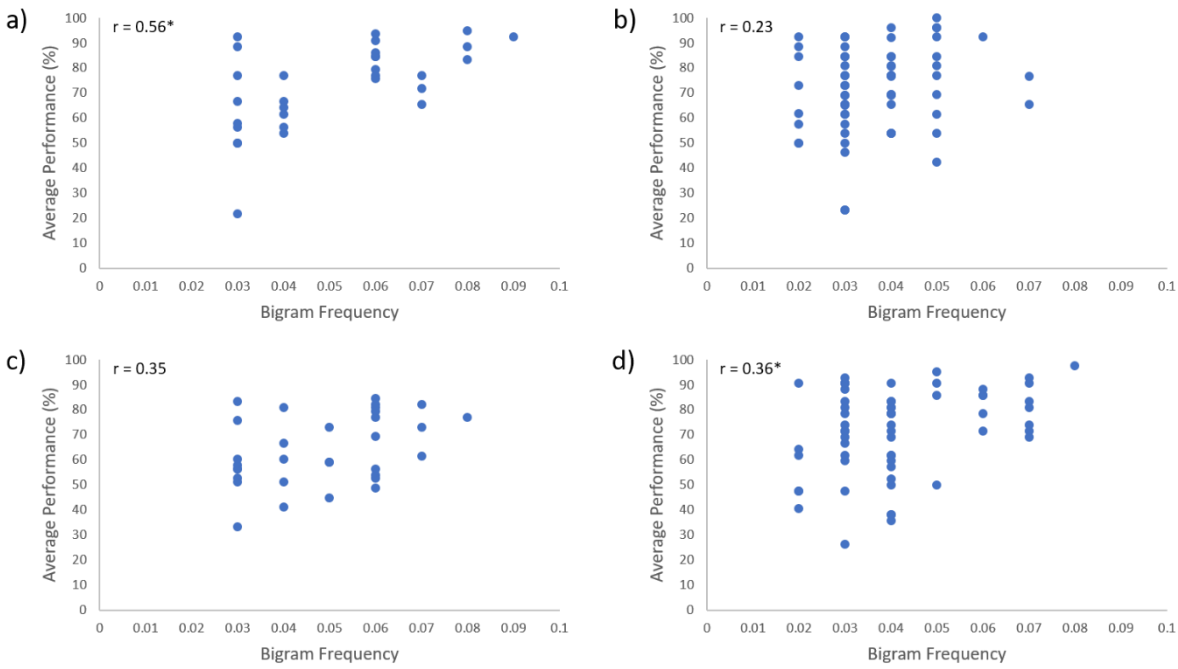

Figure 4-7: Correlations between average performance and bigram frequency.

The correlation plots for a) the NCL bird with the small vocabulary (Q32), b) the NCL bird with the large vocabulary (Q43), c) the ENTO bird with the small vocabulary (Q41), and d) the ENTO bird with the large vocabulary (Q35). * $p < 0.05$.

4.3.3. Data analysis

After filtering for visually responsive cells, a total of 135 neurons were used for data analysis, 64 from NCL and 71 from ENTO. Of the 64 visually responsive NCL neurons, 61 neurons (95.3%) were inhibitory to at least one of the stimuli, while the remaining 3 neurons (4.7%) were excitatory. Of the 71 ENTO neurons, 61 (85.9%) were classified as excitatory, and the remaining 10 neurons (14.1%) were inhibitory. Due to the extremely low numbers of inhibitory neurons in ENTO and excitatory neurons in NCL, we restricted all subsequent NCL birds' analyses to inhibitory neurons and ENTO birds' analyses to excitatory neurons.

4.3.4. Population profiles

We constructed population profiles of the excitatory neurons in ENTO birds and the inhibitory neurons in NCL birds by averaging across all words and across all nonwords for each neuron. To see if there was any difference in neural activity to the word and nonword categories, we used a two-way repeated-measures ANOVA with stimuli (words vs nonwords) and bin (6 bins for each of the periods; the middle 300 ms of the ITI, the first 300 ms of the stimulus period, and the middle 300 ms of the reward period, respectively) as factors, with repeated measures over stimuli and bins (Greenhouse-Geisser corrected). Note that bin is generally not of interest, as any significant value just reflects variations in the firing rates from one bin to the next. The population profiles are discussed in further detail in the following sections for each region.

The population profile for NCL inhibitory neurons is shown in Figure 4-8a. There was no significant main effect of stimuli in any of the three periods, all Fs < 1.46, all ps > 0.23. There was a significant main effect of bin in the reward period, $F(5,300) = 4.15$, $p = 0.03$, but not in the other two periods, both Fs < 2.23, both ps > 0.083. There was also

no significant interaction effect between stimuli and bin in any of the three periods, all *F*s < 2.46, all *p*s > 0.06.

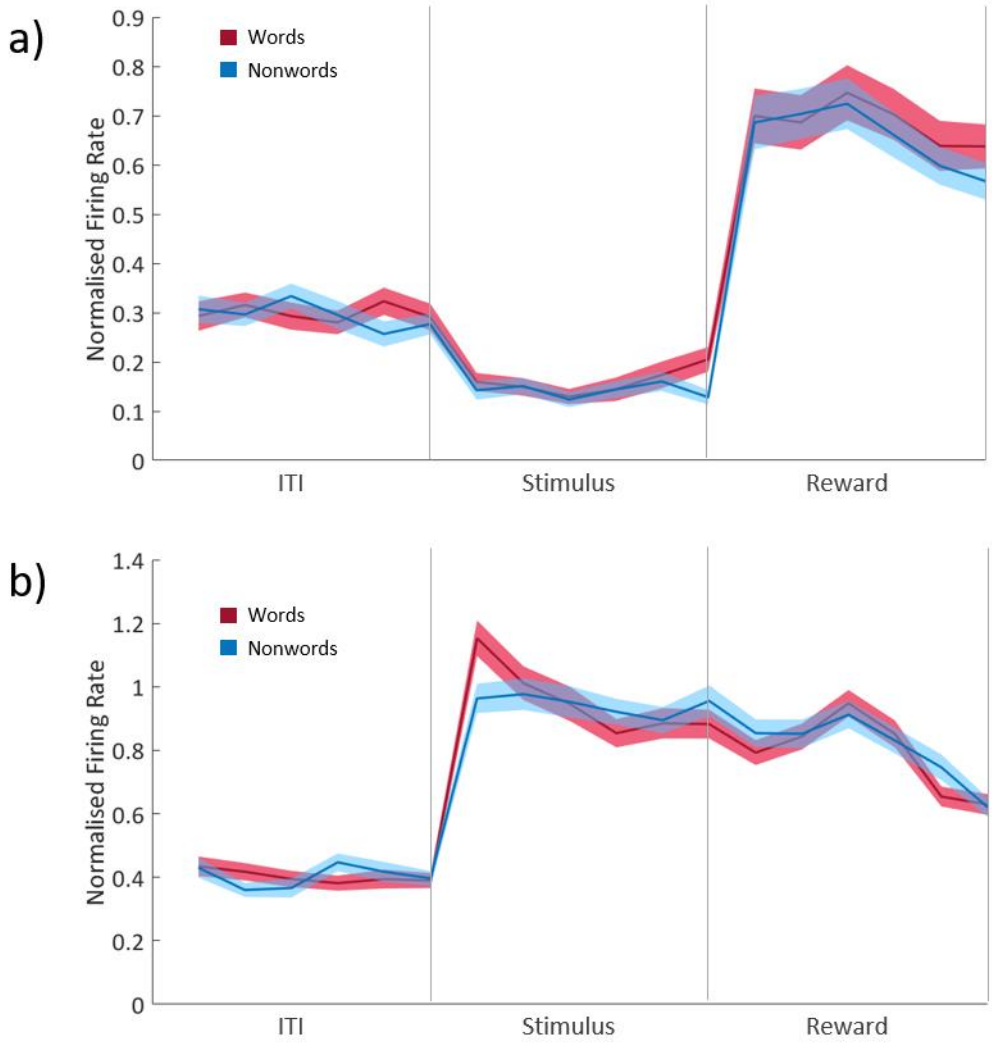

Figure 4-8: Population profiles.

Population profiles for a) NCL inhibitory neurons and b) ENTO excitatory neurons. Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval.

The population profile for ENTO excitatory neurons is shown in Figure 4-8b. There was no significant main effect of stimuli in any of the three periods, all Fs < 0.33, all ps > 0.57. There was a significant main effect of bin in both the stimulus period, $F(5,300) = 6.79$, $p < 0.001$, and the reward period, $F(5,300) = 26.80$, $p < 0.001$, but not in the ITI, $F(5,300) = 1.53$, $p = 0.18$. There was a significant interaction effect between stimuli and bin in the stimulus period, $F(5,300) = 4.58$, $p = 0.001$, with greater activity to word trials than nonwords trials at the start of the period, but with activity to word trials then decreasing across the period while activity to nonword trials remained relatively stable. There was no interaction effect between stimulus and bin in the other two periods, both Fs < 1.93, both ps > 0.097.

4.3.5. Bigram frequency differences

As a further analysis, we were interested to see whether neural activity differed between words based on their bigram frequencies. First, we removed any neurons that were not visually responsive to the word stimuli, regardless if they were visually responsive to the nonword stimuli, as we were specifically interested in activity to word trials (13 neurons from NCL birds, and 6 from ENTO birds). Then, for each bird, we calculated the bigram frequency (BF) of each word in their respective vocabularies (for more detail about how bigram frequencies are calculated, see Scarf et al., 2016). For each bird, we split their BF range into thirds – low (lower third), medium (middle third), and high (upper third). To see if there was any difference in neural activity to the words and nonwords based on BF, we used a two-way repeated-measures ANOVA with trial type (low BF, medium BF, high BF, nonwords) and bin (6 bins for each of the periods; the middle 300 ms of the ITI, the first 300 ms of the stimulus period, and the middle 300 ms of the reward period, respectively) as factors, with repeated measures over BF and bins

(Greenhouse-Geisser corrected). As each bird had a different vocabulary, and therefore different bigram frequencies, we did not collapse across birds within each area.

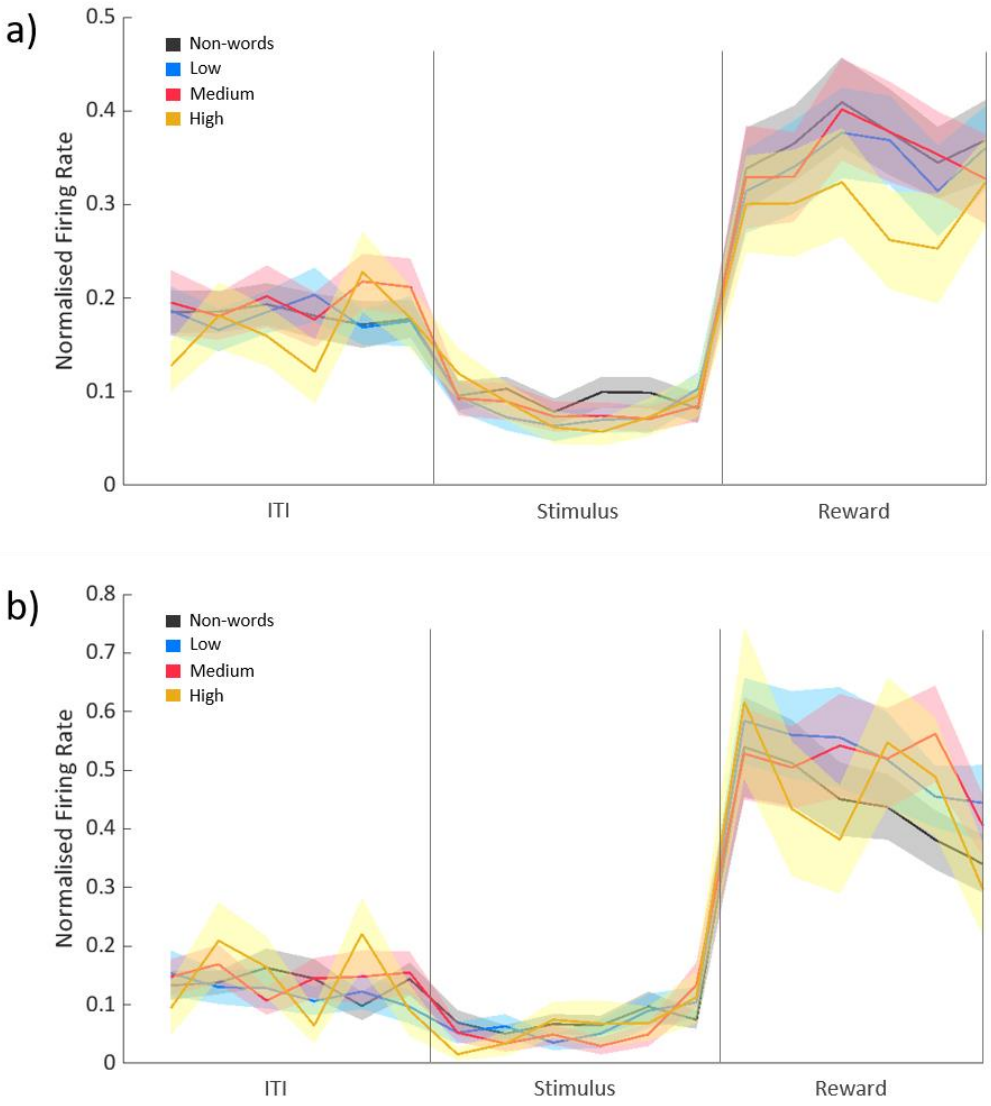

Figure 4-9: The NCL population profiles based on bigram frequencies.

The NCL population profiles based on bigram frequencies for: a) the NCL bird with a small vocabulary (Q32), and b) the NCL bird with the large vocabulary (Q43). Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval.

The population profile for the NCL bird with the small vocabulary (Q32) is shown in Figure 4-9a. There was a significant main effect of trial type in the reward period, $F(3,96) = 3.18$, $p = 0.041$, but not in the ITI or stimulus periods, both Fs < 1.84, both ps > 0.15. Post hoc pairwise comparisons using the Bonferroni correction revealed that in the reward period, activity to words with high bigram frequencies ($M = 0.18$, SE = 0.021) was significantly lower than activity to nonwords ($M = 0.21$, SE = 0.021, $p = 0.01$). Finally, there was no significant interaction effect between trial type and bin in any of the three periods, all Fs < 1.19, all ps > 0.31.

The population profile for the NCL bird with the large vocabulary (Q43) is shown in Figure 4-9b. There was no significant main effect of trial type in any of the three periods, all Fs < 1.94, all ps > 0.17. Finally, there was also no significant interaction effect between trial type and bin for any of the three periods, all Fs < 1.70, all ps > 0.14.

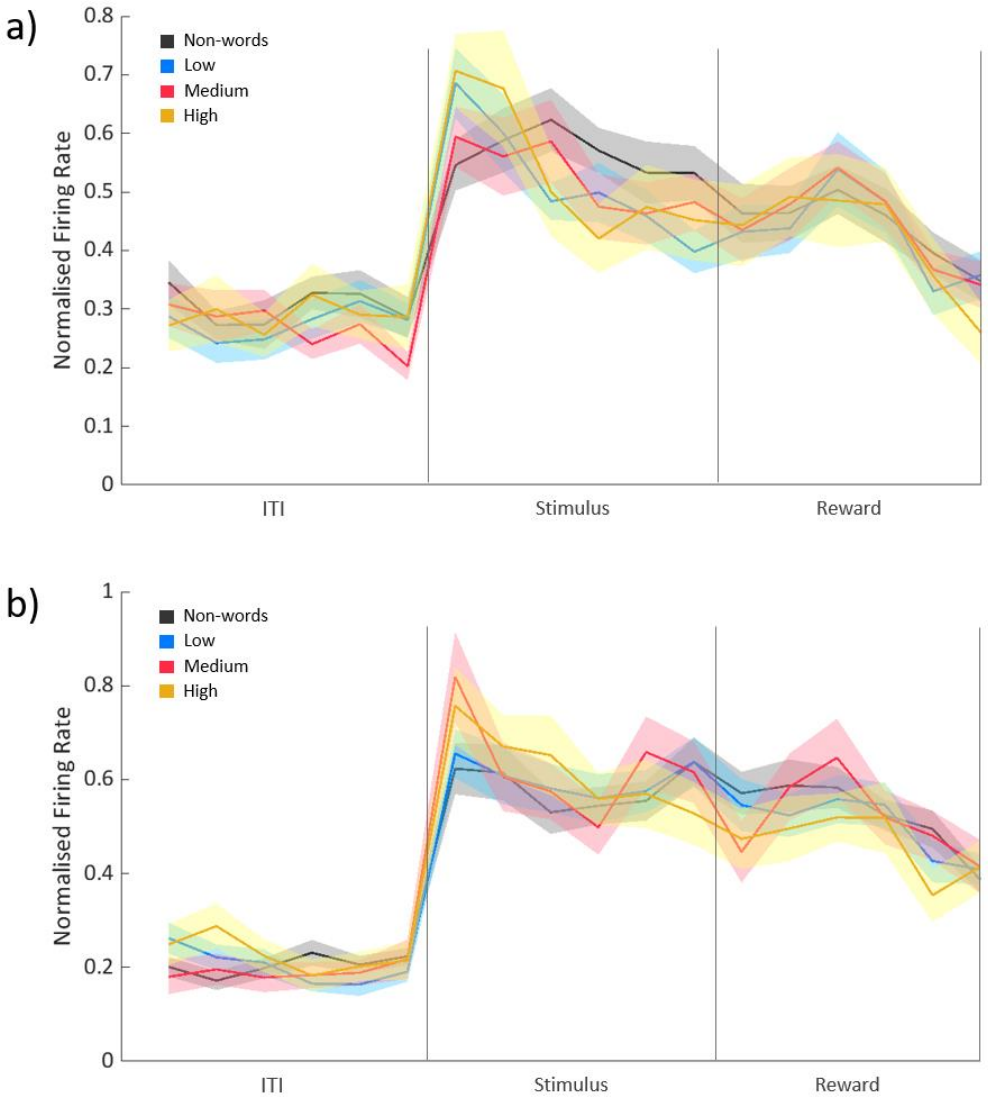

Figure 4-10: The ENTO population profiles based on bigram frequencies.

The ENTO population profiles based on bigram frequencies for: a) the ENTO bird with a small vocabulary (Q41), and b) the ENTO bird with the large vocabulary (Q35). Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval.

The population profile for the ENTO bird with the small vocabulary (Q41) is shown in Figure 4-10a. There was no significant main effect of trial type in any of the three

periods, all Fs < 1.06, all ps > 0.35. Finally, there was no significant interaction effect between trial type and bin in any of the three periods, all Fs < 1.95, all ps > 0.054.

The population profile for the ENTO birds with the large vocabulary (Q35) is shown in Figure 4-10b. There was no significant main effect of trial type in any of the three periods, all Fs < 1.63, all ps > 0.19. Finally, there was no significant interaction effect between trial type and bin in any of the three periods, all Fs < 1.43, all ps > 0.19.

4.4. Discussion

4.4.1. Summary of findings

We analysed a total of 135 visually responsive neurons, 64 from NCL and 71 from ENTO. In NCL, the majority of neurons were inhibitory (95.3%), while the majority of neurons in ENTO were excitatory (85.9%). These visually responsive neurons were further analysed using a two-way repeated-measures ANOVA (Greenhouse-Geisser corrected) to see whether neural activity differed between words and nonwords within each task period. In both NCL and ENTO, we found that overall activity to words and nonwords did not differ within any period, although in ENTO activity to words in the stimulus period decreased over time, while activity to nonwords remained relatively stable. In a further analysis, we split word trials based on bigram frequencies (low, medium, and high) and compared these with nonword trials. For one bird (Q32), we found a significant difference between activity to nonwords and activity to words with a high bigram frequency during the reward period. Differences in activity between different bigram frequencies and nonwords in the other three birds were not found.

4.4.2. Reward-based categorisation in NCL?

We predicted that NCL activity would be encoding categorical information based on behavioural outcomes such as reward. The fact that we did not find any differences between words and nonwords during the stimulus period (when the string and asterisk were presented) suggests that as expected, the NCL does not use visual properties of a stimulus to categorise objects. However, we also did not find any differences in between words and nonwords during the reward period. In the DMS studies discussed in Chapter 3, NCL appears to default to reward coding when there is the opportunity to code differential rewards, i.e. reward is different between the two stimuli (Johnston et al., 2017a). However, when reward is the same for both stimuli, NCL is able to switch to coding the to-be-remembered stimulus (Johnston et al., 2017b). In the current study, reward was the same for correctly categorising both words and nonwords, so it is likely that a lack of difference in activity during the reward period is due a lack of differential reward.

However, the categorisation study performed by Kirsch et al. (2009) found that even though correct responses to both categories resulted in the same reward, NCL neurons still encoded these rewards differently between categories. Since there was no difference in activity to overall word and nonword trials, it does not seem that NCL inhibitory neurons were able to differentiate between rewards associated with different categories. However, we did find some differences when word trials were split by bigram frequency. For the bird with the small vocabulary (Q32), we found a significant difference in activity to nonwords and words with a high bigram frequency in the reward period. A high bigram frequency indicates that a particular word contains bigrams which occur frequently in the pre-existing vocabulary. In humans, the higher the bigram frequency, the more easily a

word is learned (Broadbent & Gregory, 1968; Rumelhart & Siple, 1974). In the current study, differences in activity to words with high bigram frequencies and nonwords may be a reflection of the bird's ability to use bigram frequencies to distinguish very 'wordy' words from nonwords. The fact that we see this difference in the reward period may indicate that the bird is encoding the same reward differently between these two categories, as in Kirsch et al. (2009). On the other hand, words with medium or low bigram frequencies may be more difficult to distinguish from nonwords, as these words contain fewer common bigrams.

It is interesting to note that we only found this difference in the bird with the smallest vocabulary and not in the bird with the largest vocabulary (Q32 and Q43, 30 and 62 words, respectively). In humans, larger vocabularies increase the likelihood of encountering bigrams found in previously learned words, which elicits faster and more accurate responses than smaller vocabularies (El-Nasan et al., 2001; Mohri et al., 1998). In the current study, we did not see any evidence of using bigram frequencies to distinguish words from nonwords in the neural activity of Q43. This is also reflected in the behavioural data, with Q32 displaying a significant correlation between behavioural performance and bigram frequency, while Q43 did not. We are uncertain as to why a larger vocabulary did not appear to elicit the use of bigram frequency to guide categorisation or aid behavioural performance. Perhaps the fact that Q32 learned fewer words makes bigram frequency more useful for distinguishing words from nonwords than for Q43. All four of the birds were given the same amount of training sessions, yet two of the birds (Q43 and Q35) learned approximately twice as many words as the other two birds (Q32 and Q41) in that time. It is possible that the difference in the number of words learned may indicate better general memory in the birds with the larger vocabularies, and

thus those birds do not need bigram frequency to help them categorise words from nonwords as much as the birds with a poorer memory. We discuss memorisation further in section 4.4.4.

4.4.3. Visual-based categorisation in ENTO?

We predicted that ENTO activity would be encoding categorical information based on the visual differences between words and nonwords, which would likely be reflected by differences in bigram frequency. The fact that we did not find any differences between words and nonwords during the stimulus period (when the string and asterisk were presented) suggests that contrary to expectations, neurons in ENTO are not coding differences in the visual properties of words and nonwords. While there was a larger increase in activity to words trials over nonword trials at the very start of the stimulus period, this was not significant as activity quickly decreased to the same level as nonword trials for the remainder of the stimulus period.

A lack of difference in ENTO activity between word and nonwords trials may be caused by the fact that we cannot be sure where the bird is looking at the start and the end of the trial. In the current study, birds were presented with a four-letter string (either a word or nonword) in the centre of the screen and an asterisk symbol above or below the string simultaneously. If the string was a real word, they were required to peck the string, and if it was a nonword, they were required to peck the asterisk. We assume that the last thing a bird would be looking at would be the stimulus they are about to peck, and because a four-letter string and an asterisk are very visually different, we then also expect that neurons will be encoding the visual differences between these two stimuli. However, at the start of the stimulus period, birds must look at the four-letter string to determine which response to make (peck string or asterisk). It is likely that neural activity to the birds' first

perception of the four-letter string would be more informative as to whether neurons in ENTO are encoding visual differences between words and nonwords. It is possible that the initial difference in activity seen at the start of the stimulus period is when the birds are initially viewing the four-letter string, and thus neurons are coding visual differences. Unfortunately, we were restricted to using the 300 ms period before the peck to the sample stimulus as we cannot be sure that the bird is looking at the stimuli at the start of the trial, as opposed to some other part of the operant chamber.

We also failed to find any neural evidence of categorisation based on visual differences when we further split word trials by bigram frequency. For both birds, there is a spike in activity at the start of the stimulus period for two of the four trial types, as found in the overall word versus nonword analysis. For the bird with the larger vocabulary (Q35) greater activity occurs to words with high and medium bigram frequency, with activity to low bigram frequency words and nonwords being much lower. For the bird with the smaller vocabulary (Q41), greater activity occurs to words high and low bigram frequency, rather than words with medium bigram frequencies and nonwords. However, as with the overall analyses, activity then decreases rapidly to the same level across all four trial types in both birds. Behavioural data appears to indicate that the birds are using bigram frequencies to guide categorisation, as there was a significant correlation between bigram frequency and behavioural performance in the bird with the larger vocabulary and approached significance in the bird with the smaller vocabulary. Again, although the differences in activity at the start of the stimulus period may be more informative about what is being encoded during categorisation, and whether bigram frequency is used to guide categorisation, we were unable to analyse the start of the stimulus period.

4.4.4. Disparities between current and previous behavioural data

One main difference between the behavioural data presented in Scarf et al. (2016) and the current study was the correlations between correctly categorising words and the bigram frequency of the word. Scarf et al. (2016) found significant positive correlations between birds' behavioural performance and bigram frequency in three out of four birds (all r^2 values between 0.65 and 0.69, all ps < 0.05), with the exception of Q35 ($r^2 = 0.08$, $p = 0.40$). However, in the current study, we found that only two out of the four birds had a significant correlation between behavioural performance and bigram frequency, including Q35 (Q32: $r = 0.56$, $p = 0.01$; Q35: $r = 0.36$, $p = 0.004$), although Q41 also approached significance ($r = 0.35$, $p = 0.051$).

One reason why we may have found weaker or insignificant correlations between behavioural performance and bigram frequency in the current study may be due to memorisation. The data presented in Scarf et al. (2016) covers most of the birds training period (up to between 26 and 58 words), and thus their data covers the period in which the birds are still learning new words. In the current study however, we had since trained the same birds to between 30 and 62 words, and then kept the birds at this level for subsequent testing sessions. That is, in the current study, the behavioural data presented covers the period after learning. It may be that bigram frequency is more useful to the birds when learning new words, but as words are learned, birds are able to categorise words based on their familiarity with a word (i.e., memorisation). It is not impossible for birds to be able to memorise up to 62 words and then discriminate them from a large pool of nonwords, as previous studies have shown the long-term memory capacity of pigeons to be quite large (Cook, Levison, Gillett, & Blaisdell, 2005; Vaughan & Greene, 1984). If birds were memorising their respective vocabularies, then they would not need to rely

on orthographic knowledge such as bigrams during categorisation. However, in humans, there is mixed evidence as to whether bigram sensitivity precedes reading performance, or vice versa (for an overview see Rothe, Schulte-Körne, & Ise, 2014; Schmalz, Moll, Mulatti, & Schulte-Körne, 2019). Ultimately though, neural activity at the time of categorisation (i.e. when the stimulus is presented), should not be affected by whether our birds were using orthographic knowledge or memorisation to discriminate words from nonwords.

CHAPTER 5 | CATEGORISATION OF MONET AND PICASSO PAINTINGS

Anderson, C., Parra, R. S., Chapman, H., Steinemer, A., Porter, B., & Colombo, M. (2020). Pigeons nidopallium caudolaterale, entopallium, and mesopallium ventrolaterale neural responses during categorisation of Monet and Picasso paintings. *Scientific Reports*, *10*, 15971. (Copyright permission granted by the Creative Commons licence: http://creativecommons.org/licenses/by/4.0/)

Author contributions: CA conducted the experiment, analysed the data, and wrote the manuscript. RSP assisted with designing the experiment and assisted with collecting and analysing data. HC assisted with collecting and analysing the data. AS assisted with collecting and analysing the data. BP wrote the analysis software. MC assisted with designing the experiment and assisted with analysing the data and revising the manuscript.

In this Chapter, the second of four categorisation studies will be presented, in which birds are trained to discriminate a set of Monet paintings from a set of Picasso paintings. In the previous study, we found that the semi-symbolic paradigm was limited in that we were unable to properly compare neural activity between the two categories (see section 4.4.). In order to rectify this limitation, we used an S+/S- paradigm in the current study instead of the semi-symbolic task, in which half the birds were trained to peck Picasso paintings and refrain from responding to Monet paintings, and vice versa for the other half of the birds. For the current study, we used single-unit electrophysiology to record neural

activity in NCL, ENTO, and MVL during the Picasso/Monet discrimination task. The majority of this Chapter is taken directly from the publication above, particularly the Methods and Results sections. The Introduction and Discussion have been amended in order to better integrate the publication into the thesis.

5.1 Introduction

5.1.1. Categorisation of paintings by birds

In what is perhaps one of the classic pigeon categorisation studies, Watanabe, Sakamoto, and Wakita (1995) trained pigeons to discriminate between Picasso and Monet paintings using an S+/S- discrimination paradigm. Half of their pigeons were trained to only peck Picasso paintings (Picasso S+), and half were trained to only peck Monet paintings (Monet S+), with each group having as their S- stimuli the paintings from the opposite artist. Watanabe et al. (1995) found that pigeons successfully discriminated between Monet and Picasso paintings even when colour, contour, and sharpness were controlled for. Watanabe et al. (1995) also tested the pigeons with mirrored and upside-down versions of the stimuli and found that the pigeons were still as accurate as identifying Picasso paintings, but accuracy to Monet paintings was reduced. Most strikingly, Watanabe et al. (1995) found that birds were able to generalise to both novel instances of Picasso and Monet paintings, as well as other cubist and impressionist artists such as Matisse and Cezanne. Birds also successfully learned a pseudo-discrimination task, in which half the Picasso paintings and half the Monet paintings were grouped and presented as the S+ stimuli. Thus, Watanabe et al. (1995) argued that the pigeons were not only able to discriminate between the two artists' paintings, but also able to discriminate between individual paintings.

5.1.2. Hemispheric differences during categorisation in birds

Interestingly, general visual memory, object processing, and visual categorisation in non-human vertebrates have been shown to have a left-hemisphere dominance (Vallortigara, 2000). Birds in particular have been shown to use their right eye/left hemisphere to aid in discriminating visual stimuli, while the left eye/right hemisphere is

more involved in predator detection (chickens: Daisley, Mascalzoni, Rosa-Salva, Rugani, & Regolin, 2008; quails: Valenti, Sovrano, Zucca, & Vallortigara, 2003; zebra finches: Alonso, 1998; corvids: Clayton & Krebs, 1994; and pigeons: Güntürkün & Kesch, 1987; von Fersen & Güntürkün, 1990). It seems that the left hemisphere is more involved in identifying local features, uses category-based discrimination, and appears to be where task contingencies are stored, while the right hemisphere relies on configuration and exemplar-based discrimination (Vallortigara & Andrew, 1994; Yamazaki, Aust, Huber, Hausmann, & Güntürkün, 2007). In birds, these hemispheric asymmetries occur because embryos receive asymmetrical light stimulation during ontogeny, which causes left-dominant asymmetrical projections along the tectofugal pathway to be strengthened (Manns & Ströckens, 2014).

5.1.3. Anatomy and function of the mesopallium ventrolaterale (MVL)

We decided to record neural activity from the mesopallium ventrolaterale (MVL) as well as NCL and ENTO in the current study. MVL is one of four higher-order visual association areas that receives topographically organised projections from ENTO (see Figure 5-1; Ahumada-Galleguillos, Fernández, Marin, Letelier, & Mpodozis, 2015; Krützfeldt & Wild, 2005; Stacho et al., 2016). Located in the external layer of the mesopallium ventral (MV), the MVL is comprised of densely packed cells, which tend to be larger than those found in ENTO (Ahumada-Galleguillos et al., 2015).

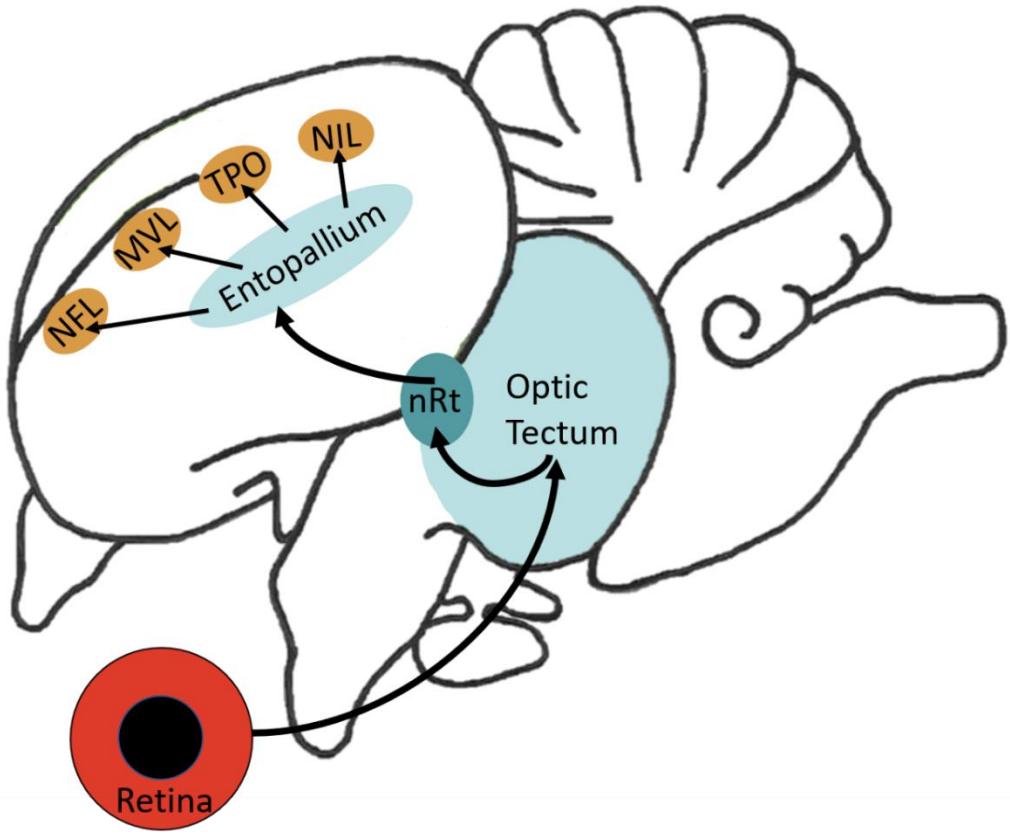

Figure 5-1: The visual association areas of the tectofugal pathway.

The tectofugal pathway runs from the retina to the entopallium. The entopallium then further projects to four higher-order visual association areas: the nidopallium frontolaterale (NFL), the mesopallium ventrolaterale (MVL), area temporo-parieto-occipitalis (TPO), and the nidopallium intermediale pars lateralis (NIL). nRt: nucleus rotundus.

In terms of function, the MVL has been compared to primate visual extrastriate cortex areas such as V2 and V4, as it is involved in the combined processing of form, colour, and motion (Stacho et al., 2016). A few recent studies have examined the role of MVL and other higher-order visual association areas in avian cognition. Koenen et al. (2016) recorded neural activity from another higher-order visual association area, the

nidopallium frontolaterale (NFL), while allowing birds to passively view and categorise stimuli that differed in colour, shape, frequency, and amplitude. Koenen et al. (2016) found that NFL neurons categorically clustered stimuli based on their features, despite the fact that there were no behaviourally defined categories for the birds to learn.

In a more recent study, Azizi, Pusch, Koenen, Klatt, Bröker, Thiele, Kellermann, Güntürkün, and Cheng (2019) employed a similar technique in which pigeons were trained to peck pictures of real-world objects to receive reward. While the pictures could be divided into animate and inanimate objects, human and non-human objects, and artificial and natural stimuli, the birds were not trained to discriminate between the stimuli. Azizi et al. (2019) found that MVL neurons discriminated between animate and inanimate objects, while ENTO neurons did not, suggesting that MVL encodes more information about the visual properties of a stimulus than neurons in ENTO. Furthermore, Azizi et al. (2019) argue that MVL neurons are more sensitive to low-level features but are also able to distinguish stimuli on a more abstract level than ENTO neurons.

5.1.4. Aims and objectives of the study

The aim of the current study was to investigate how the categories of 'Picasso' and 'Monet' are represented in NCL, ENTO, and MVL, and the involvement of these areas in the categorisation process. Based on previous categorisation studies outlined in sections 1.2.3. and 1.2.6., we expect that NCL will encode categorical information based on similar stimulus-reward associations (or rather in the case of the current study, category-reward associations), while ENTO will encode category-specific information based on visual information i.e. the visual properties of the stimuli within a category. On the basis of the few studies examining the neural activity of higher-order visual association areas outlined in the previous section, we expect that MVL will encode

categorical information in a similar yet more complex manner than in ENTO. In the current study we will also explore hemispheric differences by recording from both the left and right hemispheres in each of NCL, ENTO, and MVL.

5.2. Materials and Methods

5.2.1. Subjects

The subjects were eight experimentally naïve adult homing pigeons (*Columba livia*), and four experimentally sophisticated adult homing pigeons (*Columba livia*). Birds that were experimentally sophisticated had previously served in various studies, including a delayed matching-to-sample task, a serial-order task, and a magnetic study.

5.2.2. Apparatus and stimuli

The Perspex panel that was situated in front of the monitor had six square holes measuring 60 x 60 mm, arranged in a two (row) by three (column) grid. The holes were 65 mm apart, from centre to centre. Stimuli were presented in the top-middle square only and appeared against the white background of the monitor. There was a total of 14 different stimuli, 7 of which were Monet paintings, and 7 which were Picasso paintings. All of the paintings used were taken from the list of paintings used in the behavioural study by Watanabe et al. (1995; Monet paintings: '*Garden at Sainte-Adresse*', '*The Dinner*', '*Camille Monet and a Child in the Artist's Garden in Argenteuil*', '*Field of Poppies*', '*Boulevard of Capucines*', '*Still Life with Pears and Grapes*', '*Poplars at Giverny*'; Picasso paintings: '*The Young Ladies of Avignon*', '*Man with a Violin*', '*Guitar, Bottle and Fruit Bowl*', '*Artist and his Model*', '*Bather with Beach Ball*', '*Jug, Candle and Enamel Pan*', '*The Women of Algiers'*). The paintings were in black and

white and had been cropped from one corner so as to best preserve the essence of the original painting. Images were then resized to 100 x 100 pixels.

5.2.3. Behavioural task

The birds were trained and tested on an S+/S- discrimination task. Half of the birds were trained to peck at Picasso paintings (Picasso S+ group: B2, B10, B5, C8, D10, and D11) and the other half were trained to peck at Monet paintings (Monet S+ group: B9, B11, C3, C4, D12, and D14). Naïve and experienced birds were balanced across the groups. Depending on which S+ group the birds were assigned to, paintings from the non-S+ artist served as that birds' S- stimuli. That is, Picasso S+ birds were also Monet S-, and Monet S+ birds were also Picasso S-.

Figure 5-2 shows the sequence of events on both S+ and S- trials. Trials began with an intertrial interval (ITI) that lasted 5000 ms. At the end of the 5000 ms period, a 'ready' stimulus consisting of a small black dot appeared on the screen. This ready stimulus would remain on the screen until the bird pecked it three times. After the third peck to the ready stimulus, the dot disappeared, and a pause period began in which nothing was displayed on the screen. The pause period required birds to refrain from pecking for 1000 ms in order to continue with the trial; pecks before the 1000 ms pause period elapsed reset the timer and continued in that manner until the bird had refrained from pecking for 1000 ms.

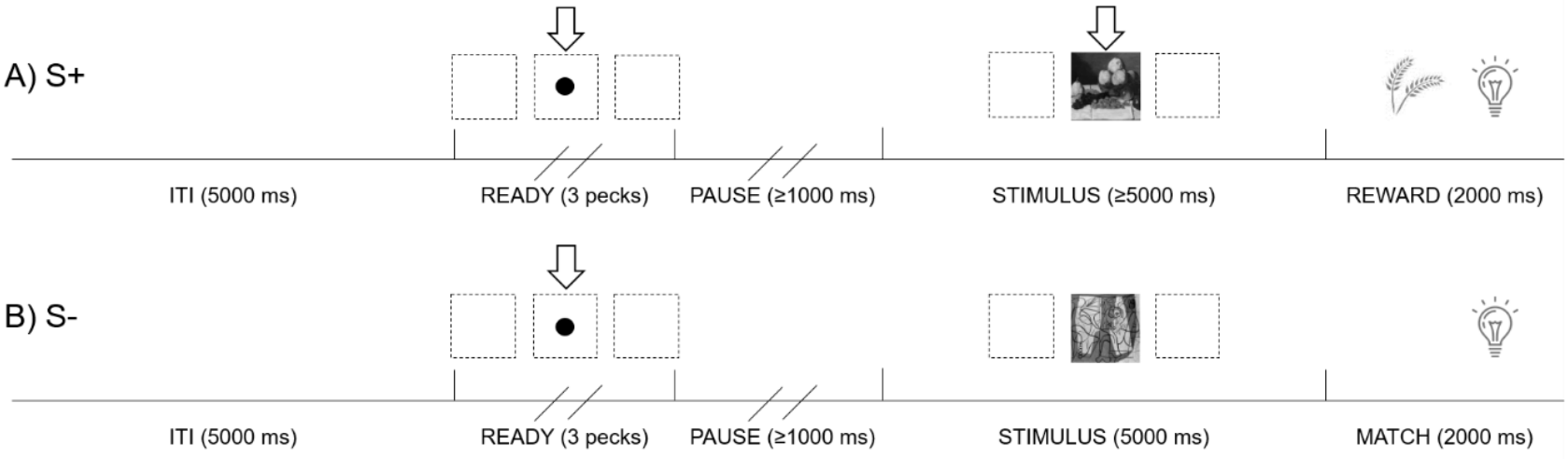

Figure 5-2: The behavioural task.

The sequence of events during an S+ trial (A) and an S- trial (B). Both trials began with a 5000 ms intertrial interval (ITI), followed by the ready period in which a black dot appeared on the screen. After pecking the dot three times, the pause period was initiated in which the birds were required to refrain from pecking for at least 1000 ms. Following the pause period, one of the 14 painting stimuli appeared for a minimum of 5000 ms, during which time pecks were recorded. If an S+ stimulus was displayed, the birds were rewarded following the first peck after 5000 ms, and the stimulus turned off. If an S- stimulus was displayed, they were required to wait 5000 ms until the stimulus disappeared automatically and were not rewarded. Wheat reward was delivered by a food hopper for 2000 ms on S+ trials, and S- trials had a matching 2000 ms period with no reward.

Once the pause period ended, one of the fourteen stimuli would appear for at least 5000 ms. If the stimulus was the S+, the first peck after 5000 ms would result in 2000 ms access to a wheat reward delivered via an illuminated hopper. If the stimulus was S-, then the stimulus would automatically disappear after 5000 ms, followed by a 2000 ms period designed to match the length of the reward periods on S+ trials, but without food. Pecks to the S+ and S- stimuli during the 5000 ms stimulus period were recorded. After the reward period on S+ trials and the matching period on S- trials, the ITI of the next trial began. Within a session, the seven S+ and seven S- stimuli were randomly presented ten times each, resulting in a total of 140 trials per session. At the completion of each session, behavioural performance was measured using a discrimination ratio (DR), calculated by dividing the number of pecks to all seven S+ stimuli by the total number of pecks to both S+ and S- stimuli.

5.2.4. Surgery

The birds underwent microdrive surgery after they had reached a DR of at least 0.85 for two consecutive days. Four pigeons had electrodes inserted into the NCL (B2, B9, C3, and C8), four pigeons had electrodes inserted into the ENTO (B5, B10, B11, and C4), and four pigeons had electrodes inserted into the MVL (D10, D11, D12, D14). For each of these regions, two birds were Monet S+ and two were Picasso S+, and two of the birds (one Monet S+ and one Picasso S+) had microdrives installed in the left hemisphere of these regions, and the other two birds had microdrives installed in the right hemisphere. For the MVL birds, the electrodes were positioned at AP +10.5, ML ± 6.0, DV = 0.5.

5.3. Results

5.3.1. Histology

All electrode tracks were within the targeted regions as defined by Karten and Hodos (1967). Figure 5-3 shows the reconstructed tracks for all twelve birds across the three areas.

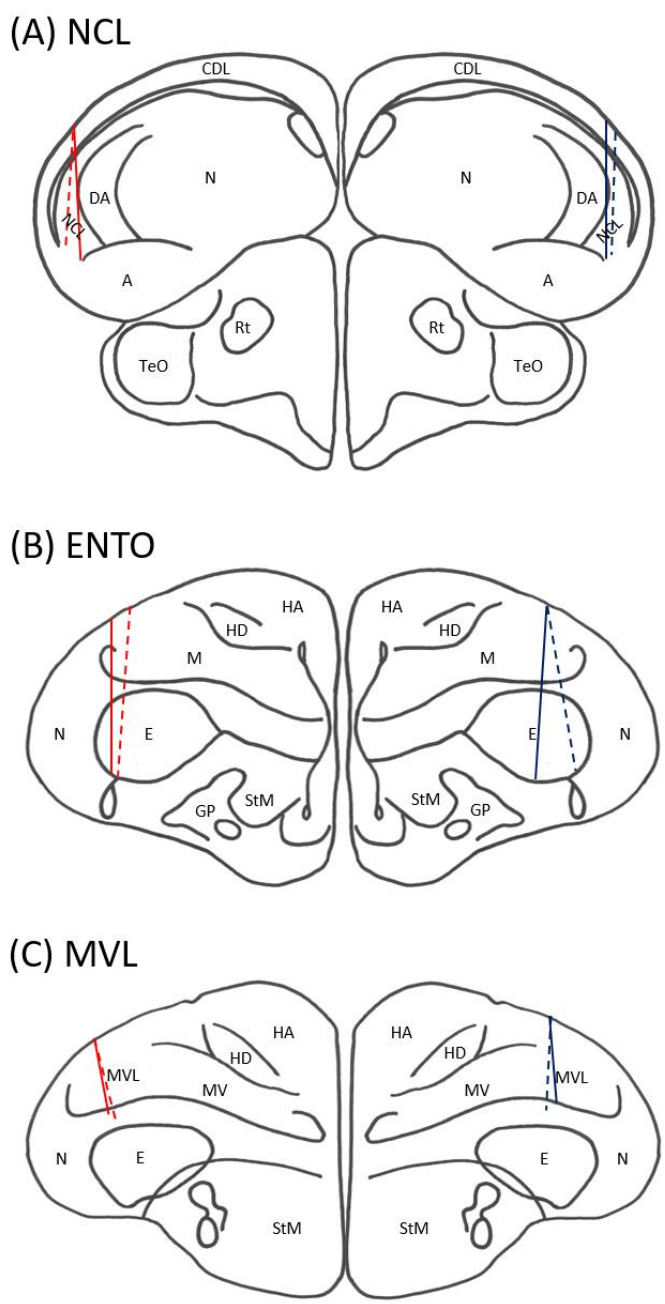

Figure 5-3: The electrode track reconstruction.

(A) NCL. Solid red line – C3; dashed red line – B2; solid blue line – B9; dashed blue line – C8. (B) ENTO. Solid red line – C4; dashed red line – B10; solid blue line – B11; dashed blue line – B5. (C) MVL. Solid red line – D12; dashed red line – D10; solid blue line – D14; dashed blue line – D11. Brain regions (as defined by Reiner et al., 2004): A, arcopallium; CDL, area corticoidea dorsolateralis; DA, tractus dorso-arcopallialis; E, entopallium; HA, hyperpallium apicale; HD, hyperpallium densocellulare; GP, globus pallidus; M, mesopallium; MV, mesopallium ventrale; MVL, mesopallium ventrolaterale; N, nidopallium; NCL, nidopallium caudolaterale; Rt, nucleus rotundus; StM, striatum mediale; TeO, tectum opticum.

For NCL, the intended track positions were AP +5.5 and ML ±7.5. The track positions for the two left hemisphere birds (B2 and C3) were AP +6.5, ML +7.5, and AP +6.0, ML +7.5, differing from the intended AP position by 1.0mm and 0.5mm, respectively. The track position for one of the right hemisphere birds (B9) was as intended at AP +5.5, ML -7.5, while the other bird (C8) was AP +5.25, ML -8.0, differing from the intended AP by 0.25mm and the intended ML by 0.5mm.

For ENTO, the intended track positions were AP +9.5 and ML ± 6.0. The track positions for the two left hemisphere birds (B10 and C4) were AP +9.0, ML +6.0, differing from the intended AP position by 0.5mm, and AP +9.5, ML +7.0, differing from the intended ML position by 1.0mm, respectively. The track position for one of the right hemisphere birds (B5) was as intended at AP +9.5, ML -6.0, while the other bird (B11) was AP +9.0, ML -6.0, differing from the intended AP position by 0.5mm.

For MVL, the intended track positions were AP +10.5 and ML ± 6.0. The track positions for the left hemisphere birds (D10 and D12) were AP +10.5, ML +7.0, differing from the intended ML position by 1.0mm, and AP +10.75, ML +7.0, differing from the intended AP position by 0.25mm and the intended ML position by 1.0mm, respectively. The track positions for the two right hemisphere birds (D11 and D14) were both AP +10.25, ML -5.5, differing from the intended AP position by 0.25mm and the intended ML position by 0.5mm.

5.3.2. Behavioural performance

The behavioural performance across all recording sessions of both Monet S+ and Picasso S+ birds, depending on the region they were implanted in, is shown in Figure 5-4. All birds discriminated between paintings of the two artists significantly above chance levels. To see if there was any difference in performance between Monet S+ and Picasso

S+ birds, as well as any difference between regions, we used a two-way ANOVA with group (Picasso S+ and Monet S+) and region (NCL, ENTO, and MVL) as factors. There was no significant effect of region or group on behavioural performance, nor an interaction effect between the two factors, all *F*s < 1.96, all *p*s > 0.22.

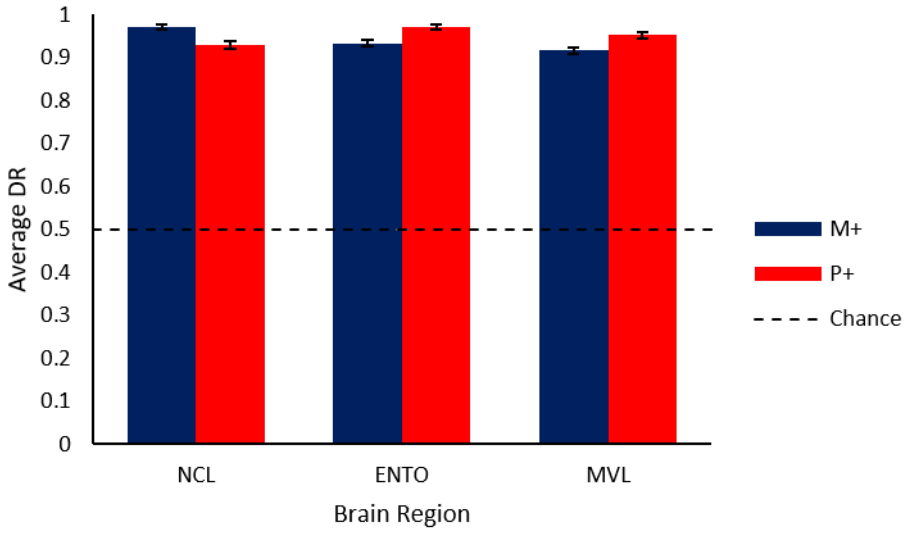

Figure 5-4: Behavioural performance.

Overall behavioural performance on the S+/S- discrimination, as indicated by the discrimination ratio. The dashed line denotes chance performance (0.5), and error bars are ±1 SEM. M+, Monet S+; P+, Picasso S+.

5.3.3. Neural filtering for visually responsive cells

Our analysis for determining whether a neuron was visually responsive in the current study differs slightly form the methods outlined in Chapter 2. First, the two-way repeated-measures ANOVA uses a 300 ms ITI period taken from the middle of the ITI rather than the middle 5s of the ITI. Secondly, the 300 ms ITI period is then compared to a 300 ms

‘stimulus first’ period. In the current study, we use the start of the stimulus period as opposed to the end as on S- trials we cannot determine whether a bird is still looking at the stimulus at the end of the 5000ms period.

Thirdly, we had to use individualised 300 ms stimulus periods for each bird. Although only the first peck to S+ stimuli after 5000 ms results in reward, all birds pecked S+ stimuli during the 5000 ms period. However, the latency to the first peck during the stimulus period varied for each bird, from approximately 300 ms to up to 1000 ms. To better capture the stimulus period for each bird, we decided to calculate the minimum average latency to the first peck to S+ stimuli during the 5000 ms period to use for further analysis. On each session that a visually responsive cell was found using the default 300 ms period, the median latency for each of the seven S+ stimuli was calculated, and then these medians were averaged across all S+ stimuli for that session. The minimum of these latencies across all sessions was rounded down to the nearest 50 ms, calculated for each bird separately. For one bird (B5), the latency remained as 300 ms; for two birds (B11 and C8) the new latency was 350 ms; for five birds (B2, B3, B9, D11, and D14) the new latency was 400 ms; for two birds (B10 and D12) the new latency was 450 ms; for one bird (D10) the new latency was 500 ms; and for the final bird (C4) the new latency was 550 ms. We then re-analysed all cells using the same ANOVA for determining if a cell was visually responsive, but with the new stimulus period lengths for each bird (stimulus periods now varied from +100 ms to +400-650 ms depending on the bird), in case the new stimulus period lengths caused any cells to no longer be classed as visually responsive or if there were cells that were previously not classed as visually responsive with the original 300 ms period. The cells that were labelled as being visually responsive from this second round of analysis were the neurons used in the final analyses.

5.3.4. Data analysis

After filtering for visually responsive cells using the new latencies for each bird, a total of 243 neurons were used for data analysis, 71 from NCL, 84 from ENTO, and 88 from MVL. Of the 71 visually responsive NCL neurons, 59 neurons (83.1%) were classed as excitatory while the remaining 12 neurons (16.9%) were inhibitory. Of the 84 ENTO neurons, 80 (95.2%) were classified as excitatory and the remaining 4 neurons (4.8%) were inhibitory. Of the 88 MVL neurons, 77 (87.5%) were excitatory and 11 (12.5%) were inhibitory. Due to the extremely low numbers of inhibitory neurons across all three areas, we restricted all subsequent analyses to just the excitatory neurons.

5.3.5. Population profiles

We constructed population profiles of the excitatory neurons for each bird by averaging across all S+ stimuli and across all S- stimuli for each neuron. To see if there was any difference in neural activity to the Picasso and Monet painting categories, we used a two-way repeated-measures ANOVA with S+S- (S+ stimuli vs S- stimuli) and bin (6 bins for each of the periods; the middle 300 ms of the ITI, the last 300 ms before the first ready peck, the middle 300 ms of the pause period, the last 300ms of each bird's specific stimulus period, and the middle 300 ms of the reward period, respectively) as within-subjects factors (Greenhouse-Geisser corrected). We used Keppel's (1982) modified Bonferroni correction ($p < 0.02$), which was calculated on the basis of ten comparisons (five ANOVAs for each of the five periods: ITI, ready, pause, sample, and reward; for both S+ and S- stimuli). The population profiles are discussed in further detail in the following sections for each region. Note that we do not report results for any main effects of bin, as any significant value just reflects variations in the firing rates from one

bin to the next, and is generally not of interest, but we do report interaction effects between S+S- and bin.

The population profile for NCL is shown in Figure 5-5a. There was no significant main effect of S+S− in any of the five periods, all Fs < 4.90, all *p*s > 0.031. There was a significant interaction effect between S+S− and bin in the reward period, $F(5,290) = 4.39$, $p = 0.004$, with activity on S+ trials increasing across the period, but not on S− trials. There was no interaction effect in the other four periods, all *F*s < 1.54, all *p*s > 0.19.

The population profile for ENTO is shown in Fig. 5-5b. There was no significant main effect of S+S− in the ITI, ready, pause, or reward periods, all *F*s < 4.73, all *p*s > 0.033. However, there was a significant main effect of S+S− in the stimulus period, $F(1,79) = 47.94$, $p < 0.001$, with activity to S+ stimuli being significantly greater than activity to S− stimuli. Finally, there was a significant interaction effect between S+S− and bin in the stimulus period, $F(5,395) = 4.43$, $p = 0.001$, with activity on S− trials decreasing across the period more rapidly than S+ trials. There was no interaction effect in the other four periods, all *F*s < 2.39, all *p*s > 0.042.

The population profile for MVL is shown in Figure 5-5c. There was no significant main effect of S+S- in the ITI, ready, pause, or reward periods, all *F*s < 0.39, all *p*s > 0.53. However, there was a significant main effect of S+S- in the stimulus period, $F(1,76) = 33.51$, $p < 0.001$, with significantly greater activity to S+ stimuli than S- stimuli. Finally, there was a significant interaction effect between S+S- and bin in the stimulus period, $F(5,380) = 3.29$, $p = 0.016$, with activity on S- trials decreasing across the period but not on S+ trials. There was no interaction effect in the other four periods, all *F*s < 2.26, all *p*s > 0.059.

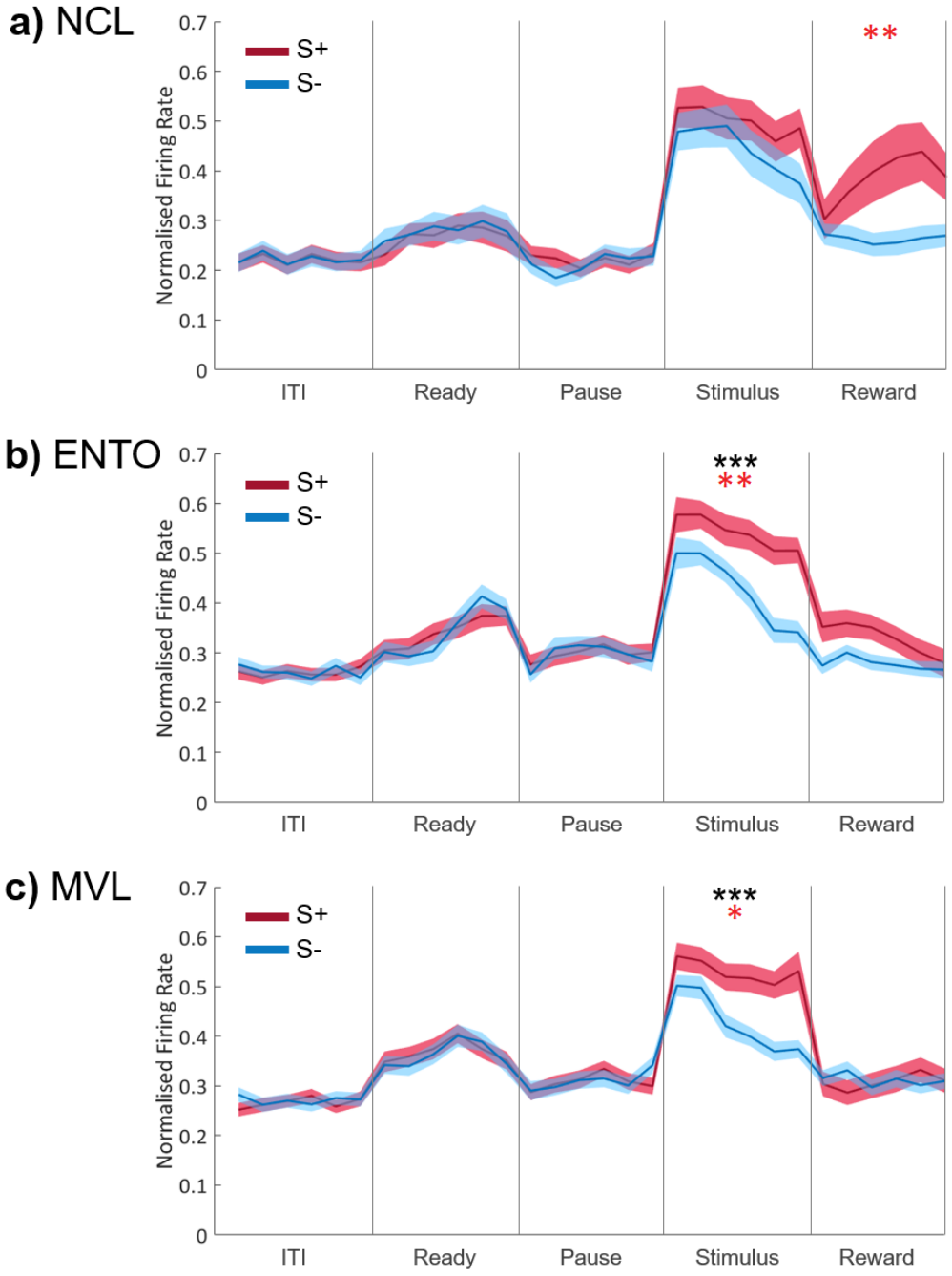

Figure 5-5: Overall population profiles.

The overall population profiles for (A) NCL, (B) ENTO, and (C) MVL. Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. Black asterisks indicate a significant main effect of S+S-, and red asterisks represent a significant interaction effect between S+S- and bin. ITI, intertrial interval. * $p < .02$, ** $p < .01$, *** $p < .001$.

5.3.6. Hemispheric differences

As a further analysis, we examined whether there were any hemispheric differences in firing patterns within each region. We used a three-way repeated-measures ANOVA with S+S- (S+ stimuli vs S- stimuli) and bin (6 bins for each of the periods; the middle 300 ms of the ITI, the last 300 ms before the first ready peck, the middle 300 ms of the pause period, the last 300 ms of each birds' specific stimulus period, and the middle 300 ms of the reward period, respectively) as within-subjects factors, and hemisphere (left vs right) as a between-subjects factor (Greenhouse-Geisser corrected). We used the same Keppel's (1982) modified Bonferroni correction ($p < 0.02$) as in the earlier overall analyses. Again, we do not report results for any main effects of bin, nor any interaction effects between bin and S+S-, as these interactions are identical to those reported from the overall analyses.

The hemispheric population profiles for left and right NCL are shown in Figure 5-6a and 5-6b, respectively. There was no significant main effect of S+S− in any of the five periods, all Fs < 5.24, all ps > 0.026, nor a significant main effect of hemisphere in any of the five periods, all Fs < 3.29, all ps > 0.075. However, there was a significant interaction effect between S+S− and hemisphere in the reward period, $F(1,57) = 10.67$, $p = 0.002$, but not in any of the other four periods, all Fs < 4.29, all ps > 0.043. To further understand the interaction effect found in the reward period, we used paired t-tests to see whether there was any difference between S+ and S− trials in each hemisphere. In the left hemisphere, there was a significant difference between S+ and S− trials, with greater activity to S+ over S− trials, $t(28) = 3.22$, $p = 0.003$. In the right hemisphere, there was no difference between S+ and S− trials, $t(29) = 1.13$, $p = 0.27$. Finally, there was no

interaction effect between bin, S+S−, and hemisphere in any of the five periods, all Fs < 2.88, all ps > 0.021.

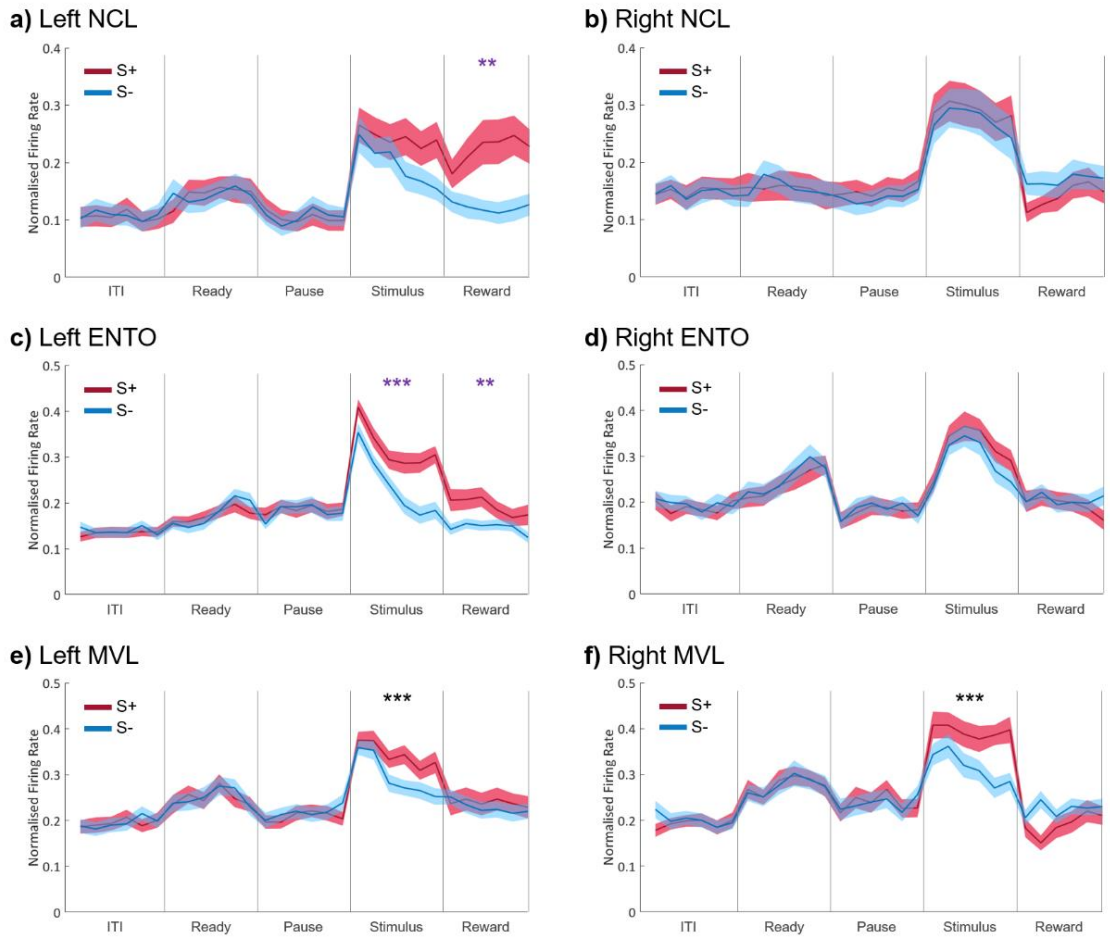

Figure 5-6: Hemispheric population profiles.

The population profiles for left (A) and right (B) hemisphere NCL, left (C) and right (D) hemisphere ENTO, and left (E) and right (F) hemisphere MVL. Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval. Black asterisks represent significant S+S- differences that are the same across hemispheres, purple asterisks represent significant S+S- differences that are specific to one hemisphere. * p < .02, ** p < .01, *** p < .001.

The hemispheric population profiles for left and right ENTO are shown in Figure 5-6c and 5-6d, respectively. There was a significant main effect of S+S− in the stimulus period, $F(1,78) = 49.84$, $p < 0.001$, but not in any of the other four periods, all Fs < 2.59, all ps > 0.11. There was a significant main effect of hemisphere in the ITI, $F(1,78) = 10.16$, $p = 0.002$, and ready period, $F(1,78) = 9.99$, $p = 0.002$, but not in the other three periods, all Fs < 2.35, all ps > 0.13. Finally, there was a significant interaction effect between S+S− and hemisphere in the stimulus period, $F(1,78) = 12.35$, $p = 0.001$, and the reward period, $F(1,78) = 5.77$, $p = 0.019$, but not in the other three periods, all Fs < 0.65, all ps > 0.42. To further understand the interaction effect found in the stimulus and reward periods, we used paired t-tests to see whether there was any difference between S+ and S− trials in each hemisphere. In the left hemisphere, there was a significant difference between S+ and S− trials in the stimulus period, $t(38) = 9.52$, $p < 0.001$, and in the reward period, $t(38) = 2.98$, $p = 0.005$, with greater activity to S+ over S− trials in both periods. In the right hemisphere, there was no significant difference between S+ and S− trials during either period, both ts < 2.16, both ps > 0.037. Finally, there was no interaction effect between bin, S+S−, and hemisphere in any of the five periods, all Fs < 1.97, all ps > 0.091.

The hemispheric population profiles for left and right MVL are shown in Figure 5-6e and 5-6f, respectively. There was a significant main effect of S+S- in the stimulus period, $F(1,75) = 44.87$, $p < 0.001$, but not in any of the other five periods, all Fs < 0.69, all ps > 0.41. There was no main effect of hemisphere in any of the five periods, all Fs < 2.00, all ps > 0.16. There was also no significant interaction effect between S+S- and hemisphere in any of the five periods, all Fs < 3.05, all ps > 0.085. Finally, there was no interaction

effect between bin, S+S-, and hemisphere in any of the five periods, all Fs < 2.19, all ps > 0.067.

5.4. Discussion

5.4.1. Summary of findings

We analysed a total of 243 visually responsive neurons, 71 from NCL, 84 from ENTO, and 88 from MVL. In each of these areas, the majority of neurons were excitatory (83.1%, 95.2%, and 87.5% of neurons in NCL, ENTO, and MVL, respectively). These excitatory neurons were further analysed using a two-way repeated-measures ANOVA (Greenhouse-Geisser corrected) to see whether neural activity differed between S+ and S- stimuli within each task period. In NCL, we found that overall activity to S+ and S- stimuli did not differ within any period. In ENTO and MVL, we found that overall activity to S+ and S- stimuli only differed within the stimulus period, in which activity to S+ stimuli was greater than activity to S- stimuli.

5.4.2. Categorisation based on reward outcomes in NCL

We predicted that the NCL would encode categorical information based on behavioural outcomes such as reward. Interestingly, we found that the overall population of NCL neurons did not distinguish between Picasso and Monet paintings in any period. However, when hemisphere was added as a factor to the analyses, we found differences in activity to S+ and S- stimuli during the reward period. Left hemisphere neurons showed greater activity during S+ trials than during S- trials, but right hemisphere neurons fired equally to S+ and S- trials. Like with visual memory, pigeons have been shown to have a left-hemisphere dominance during simple reward-related discriminations (Güntürkün,

Diekamp, Manns, Nottelmann, Prior, Schwarz, & Skiba, 2000; Güntürkün & Kesch, 1987). In fact, the left hemisphere appears to be specialised for discriminating important learned stimuli (such as food) from distractor stimuli (such as pebbles or grit), while the right hemisphere is more easily distracted by novel stimuli (Rogers, 2008; Yamazaki et al., 2007). Note that for half the birds, the S+ stimuli were Picasso paintings and for the other half they were Monet paintings, indicating that the increased activity towards S+ stimuli was not solely based on stimulus properties (e.g. all Picasso paintings are more interesting than all Monet paintings), but also on which paintings were part of the rewarded category (S+ stimuli vs. S- stimuli). We have previously shown that reward-based coding is more dominant in NCL, and that when there is the opportunity to code information in this way (due to differential reward outcomes), NCL neurons more or less default to reward coding over stimulus coding (Johnston et al, 2017a; 2017b). Therefore, as in Kirsch et al. (2009), NCL neurons are likely categorising information based on stimulus-reward associations, rather than the visual properties of the stimuli in each category.

5.4.3. Categorisation based on visual properties in ENTO

We predicted that ENTO would encode categorical information based on category-specific information, that is, visual differences between Monet and Picasso paintings. We found that the overall population of ENTO neurons only distinguished between the two categories of paintings in the stimulus period, with neural activity being significantly more excitatory towards S+ stimuli than S- stimuli. The difference in activity in the stimulus period suggests that ENTO can distinguish between the two categories of paintings, likely based on differences in the visual properties of the paintings. The idea that ENTO is an area which processes stimulus information over reward information is

consistent with the findings of many other studies (Johnston et al., 2017a; Watanabe, 1991; 1992). Furthermore, we also found a significant difference between S+ and S- trials in both the stimulus and reward periods when hemisphere was added as a factor to the analyses. In both the stimulus and reward periods, left hemisphere neurons showed greater activity during S+ than S- trials, while right hemisphere neurons fired equally to S+ and S- trials. A left-hemisphere dominance for visual categorisation in birds has been well-established (Vallortigara, 2000; Vallortigara & Andrew, 1994; Yamazaki et al., 2007), but ENTO has also been shown to have a stronger left hemisphere dominance in a colour discrimination task when differential reward outcomes were strengthened (Verhaal, Kirsch, Vlachos, Manns, & Güntürkün, 2012). Reward processing in a visual area is not unprecedented; while visual areas such as ENTO may primarily process visual/stimulus information, both ENTO and other visual areas such as the Wulst have been shown to be modulated by reward information (Anderson, Johnston, Marrs, Porter, & Colombo, 2020; Johnston et al., 2017a). Due to the nature of the S+/S- discrimination task, reward is intrinsically tied to stimulus information, causing both types of information to be useful in distinguishing between the two categories. Therefore, ENTO neurons appear to encode categorical information based on category-specific information, but that this category-specific information is not limited to visual information.

5.4.4. Categorisation based on visual properties in MVL

Finally, we predicted that MVL would encode categorical information in a similar yet more complex manner than in ENTO. Like in ENTO, we found that the overall population of MVL neurons only distinguished between the two categories of paintings in the stimulus period, with neural activity being significantly more excitatory towards S+ stimuli than S- stimuli. Similarly, we argue that MVL also encodes categorical

information based on category-specific information. Based on overall population profiles, there is little that would distinguish the coding properties of MVL from the coding properties of ENTO. Interestingly, MVL was the only area that did not display a left-hemisphere dominance; both hemispheres displayed a significant difference in activity during the stimulus period, with greater activity to S+ stimuli over S- stimuli. A lack of left hemisphere lateralisation in MVL has been previously noted by Stacho et al. (2016), in which both hemispheres were equally activated by form, colour, and motion. Also, unlike ENTO, MVL did not display any differences in activity during the reward period. Perhaps the fact that significant categorical processing only occurs in the stimulus period for both hemispheres indicates a stronger ability for MVL to categorise based on visual properties than in ENTO. MVL has been shown to be sensitive to visual features of stimuli and intrinsically categorise information based on those features (Azizi et al., 2019; Koenen et al., 2016). On the other hand, ENTO has been shown to be specifically involved in forming new categories (Watanabe, 1991; 1992; 1996), which in an S+/S- discrimination task would be greatly facilitated by incorporating reward information. ENTO lesions also cause deficits in pseudo-categorisation tasks (Watanabe, 1991), in which categorisation would rely heavily on identifying individual stimuli rather than viewing all stimuli in one category as the same. It is possible that higher-order visual association areas like MVL only use stimulus information to categorise objects, compared to ENTO which perhaps incorporates some reward information as well. While we do not directly compare the three areas to one another, our results indicate that all three areas appear to be involved in similar yet distinct steps of the categorisation process. ENTO has reciprocal projections with both NCL (Kröner & Güntürkün, 1999) and MVL (Krutzfeldt & Wild, 2005), and therefore it is likely that reward-related information about

the categories is shared between NCL and ENTO, and stimulus-related information about the categories is shared between MVL and ENTO.

5.4.5. Limitations of the study

It should be noted that while our results indicate a strong left hemisphere dominance for categorisation in both NCL and ENTO, these results should be considered as being preliminary in nature. Due to the small number of subjects, the number of neurons from each hemisphere are relatively small and may not represent the population as a whole. The small number of neurons in each hemisphere may be why we have found no hemispheric differences in MVL, and with larger number of neurons, hemispheric differences may emerge. Furthermore, due to the limits of single-unit electrophysiology, we are unable to compare neurons from both hemispheres within individual subjects, which may mean that there is a degree of individual variability within these results. However, the fact that we find such strong differences in NCL and ENTO when directly comparing hemispheres should not be discounted, especially considering previous literature which clearly shows that categorisation is lateralised to the left hemisphere in birds.

Another limitation of the current study was the use of differential reward outcomes. As previously mentioned, due to the nature of the S+/S- discrimination task, reward is intrinsically tied to stimulus information. That is, for birds who were Monet S+, Monet paintings were the only stimuli that were rewarded, and for Picasso S+ birds, Picasso paintings were the only stimuli that were rewarded. Therefore, it could be that the difference in activity on S+ and S- trials during the stimulus period could be a reflection of reward anticipation, rather than differences in visual properties. Indeed, in left ENTO, we found a significant difference between S+ and S- trials in both the stimulus period,

which then appears to continue through to the reward period. Conversely, in MVL, differences in activity to S+ and S- stimuli in the stimulus period does not continue through the reward period; instead, activity on both trial types decreases suddenly to the same level, and thus seems less likely to be due to reward anticipation. Although it is ambiguous whether differences in activity to S+ and S- stimuli reflects categorisation based on stimulus properties or reward in ENTO, we argue that the most likely possibility is that ENTO neurons incorporate both types of information during categorisation.

In conclusion, our results indicate that ENTO and MVL neurons primarily use stimulus information to discriminate between Monet and Picasso paintings, while NCL neurons are likely using reward information to drive this discrimination. However, ENTO is also able to incorporate reward information to help discriminate two categories. On a hemispheric level, we found preliminary evidence that ENTO and NCL show a strong left-hemisphere dominance, in that left hemisphere neurons categorise Monet and Picasso paintings more strongly than right hemisphere neurons. In MVL, we did not find any hemispheric asymmetries in categorising paintings, which may reflect more complex visual categorisation occurring in high-order visual areas. Overall, it is apparent that both visual and working memory areas of the pigeon brain are involved in the categorisation of Picasso and Monet paintings.

CHAPTER 6 | EXAMINING RELEVANT AND IRRELEVANT FEATURES OF CATEGORIES DURING CATEGORISATION

In this Chapter, the third of four categorisation studies will be presented, in which birds are trained to discriminate between two categories of complex 'exemplars. Each exemplar from each category is made up of four smaller stimuli, two of which are always unique to the category, and two which are shared between the categories (fixed features). In the previous study, one of the main pitfalls of the S+/S- paradigm was that the categories were associated with differential reward outcomes, in which one set of paintings was always rewarded, and the other set not (see section 5.4.5.). These differential rewards made it difficult to parse whether differences in neural activity were representative of categorisation, or simply reward anticipation. In order to rectify this limitation, we used a simultaneous matching-to-category (SMC) paradigm. In the SMC task, birds were trained to peck one of two pattern stimuli mapped onto the category that the shown exemplar belonged to and were awarded the same reward for correct responses to both categories. For the current study, we used single-unit electrophysiology to record neural activity in NCL and ENTO during the fixed features SMC task. Note that for the current study, we did not record neural activity from MVL due to time restrictions.

6.1. Introduction

6.1.1. Attention to features during categorisation

Categorisation requires an individual to attend to the features of a stimulus that signal that it belongs to a certain category (relevant features) while ignoring the stimulus features that are shared between multiple categories (irrelevant features; Castro & Wasserman, 2014). For example, both apples and oranges have a round shape, which is not useful in distinguishing between the two categories. However, apples tend to have a red or green colour, a feature which is different to oranges. Thus, children learn to attend to the colour of the fruit and ignore the shape when discriminating between apples and oranges.

An important aspect of learning which features are relevant and irrelevant to a category is the amount of attention directed towards these features. As Lawrence (1949) posited, attention is likely to be distributed evenly between all features at the start of learning as the individual has not yet learned which features are relevant or irrelevant to the category. However, as learning progresses, error feedback shapes an individual's behaviour and attention, with attention to relevant features increasing and attention to irrelevant features decreasing. In humans, a change in attention to relevant and irrelevant features has been measured through eye-tracking (Blair, Watson, Walshe, & Maj, 2009; Rehder & Hoffman, 2005) and event-related potentials during prediction errors (Wills, Lavric, Croft, & Hodgson, 2007). In animals, eye-tracking has been more difficult to implement as a means of measuring attention to stimulus features, although some eye-tracking studies have been done with primates (see Awh, Armstrong, & Moore, 2006). Studies using non-primates have used other techniques to assess selective attention, such as applying a generalised context model (GCM) in rats (Broschard, Kim, Love, Wasserman, & Freeman, 2019), and the "Bubbles" technique in pigeons (Gibson, Wasserman,

Gosselin, & Schyns, 2005). Peck rates in pigeons have also been used as a measure of attention, with higher pecks rates being associated with more attention (Pearce, Esber, George, & Haselgrove, 2008). More recently however, peck tracking in pigeons has been adopted as a more robust method for assessing selective attention in pigeons, that is, the actual locations of pigeons' pecks (see Dittrich, Rose, Buschmann, Bourdonnais, & Güntürkün, 2010).

6.1.2. Tracking pecks to relevant and irrelevant features

Castro and Wasserman (2014) tracked pigeons' pecks during a complex categorisation task, in which exemplars from two categories were made up of four smaller stimuli, two of which were unique to each category (relevant features) and two that were shared between both categories (irrelevant features). One group of birds were trained with only two irrelevant features, in that both irrelevant features were always presented in the category exemplars (Fixed Irrelevant group; FI). Another group were trained with eight different irrelevant features, in that when category exemplars were presented, two of the eight irrelevant features would be randomly presented in the exemplars (Variable Irrelevant group; VI). Each category was associated with a particular response key, and birds were able to peck any of the four smaller stimuli that comprised the overall exemplar between 5 and 15 times before selecting which category an exemplar belonged to. Birds alternated between training and testing sessions, with only difference between session being that birds were always rewarded for their responses on testing sessions, regardless of whether the response was correct or incorrect.

Castro and Wasserman (2014) found that both groups reached 85% accuracy on two consecutive days after an average of 30 days, with the FI group learning the task slightly quicker than the VI group. In both groups, birds initially distributed their pecks amongst

the four stimuli equally, but by the end of training both groups pecked relevant stimuli more often than irrelevant stimuli (Figure 6-1, left). Peck location was also able to predict behavioural accuracy, with higher behavioural performance following pecks to relevant stimuli, and lower behavioural performance following pecks to irrelevant stimuli (Figure 6-1, right). Castro and Wasserman (2014) argue that both the change in pecking during training and the fact that peck locations were able to predict behavioural accuracy demonstrates that peck tracking is a good measure of attention in pigeons, and that pigeons had learned to attend to relevant features while simultaneously learning to ignore irrelevant features.

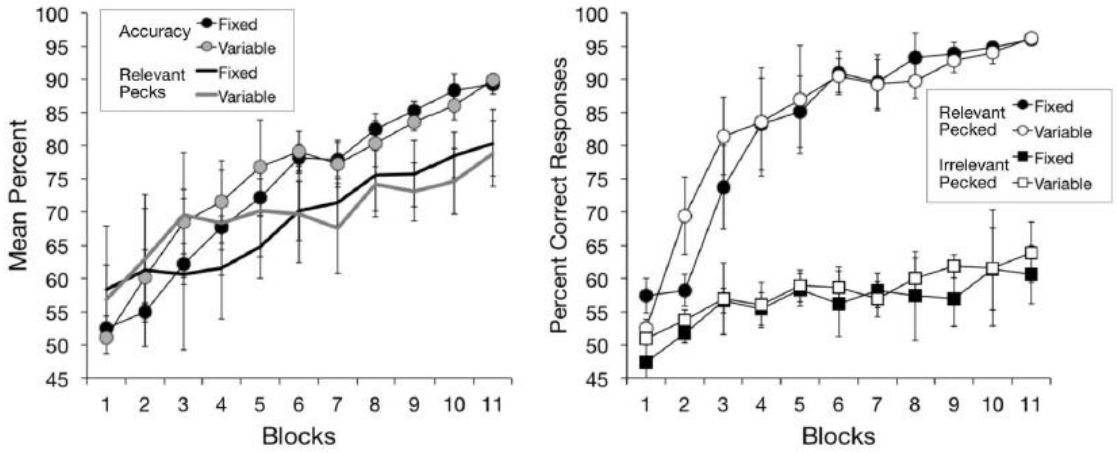

Figure 6-1: Behavioural data from Castro and Wasserman (2014).

Left: Average behavioural performance and pecks made to relevant stimuli during training, for both Fixed Irrelevant and Variable Irrelevant groups. Right: Average behavioural performance during training depending on whether birds pecked relevant or irrelevant features, for both groups. All error bars are ±1 SEM.[6]

[6] Copyright © 2014 by American Psychological Association. Reproduced with permission. Castro, L., & Wasserman, E. A. (2014). Pigeons' tracking of relevant attributes in categorisation learning. *Journal of Experimental Psychology: Animal Learning and Cognition*, *40*(2), 195-211. https://doi.org/10.1037/xan0000022

Further evidence that the birds learned to specifically ignore irrelevant stimuli comes from a second experiment, in which the relevant features in each category were replaced with novel relevant features. The birds learned the second task more quickly than in the first experiment, indicating that birds continued to ignore irrelevant stimuli to solve the discrimination. In a third experiment, Castro and Wasserman (2014) reversed the associated response keys for each category. They found that the performance of both FI and VI birds dropped to chance levels, but that both groups were able to re-learn the task. Most interestingly, while FI birds returned to pecking relevant stimuli at a much higher rate than irrelevant stimuli, VI birds pecking rate to relevant stimuli did not increase to the same rate as in the first experiment. However, VI birds still reached a similar level of behavioural accuracy, indicating that while the relevancy of features are the best predictors for behavioural accuracy, pecking relevant features is not necessary to solve the discrimination.

6.1.3. Aims and objectives of the study

The aim of the current study was to investigate how two categories of complex stimuli with varying features are represented in NCL and ENTO, and the involvement of these areas in the categorisation process. Again, based on previous categorisation studies outlined in sections 1.2.3., 1.2.6., and 5.1.3., we expect that NCL will encode categorical information beyond the visual properties of the stimuli, i.e. an amalgamation of both behavioural and category-reward associations. On the other hand, we expect that ENTO will encode category-specific information based on visual information i.e., the visual properties of the stimuli within a category. In the current study we will also explore how pigeons attend to both relevant and irrelevant features of stimuli during categorisation, and how relevancy is represented neurally. Relevancy appears to be an important tool for

guiding behaviour in Castro and Wasserman (2014), as behavioural accuracy was able to be predicted by whether the birds pecked relevant or irrelevant features. Thus, since relevancy is linked to guiding behavioural decisions, we expect that NCL will be involved in how relevancy is coded neurally. On the other hand, we expect ENTO to be more involved at the individual stimulus level, that is, how the visual features of the four different stimuli that make up and exemplar are represented neurally.

We use the same stimuli from the FI group in Castro and Wasserman (2014), as well as a non-differential reward paradigm (simultaneous matching-to-category; SMC) similar to the one used by Castro and Wasserman (2014), in order to eliminate the possibility that the differences in neural activity to the categories is simply a reflection of reward anticipation as in the S+/S- paradigm used in Chapter 5. Based on the findings of Castro and Wasserman (2014), we also expect that behaviourally, our birds will learn to attend to relevant features and ignore irrelevant features during categorisation.

6.2. Materials and Methods

6.2.1. Subjects

The subjects were eight experimentally naïve adult homing pigeons (*Columba livia*).

6.2.2. Apparatus and stimuli

In front of the monitor was a Perspex panel with one large rectangular hole measuring 245(w) x 105(h) mm. The whole experimental configuration was displayed within this singular hole. We used the same 24 Category A and 24 Category B exemplar stimuli from the Fixed Irrelevant Group in Castro and Wasserman (2014). That is, each of the exemplars were made up of four features, two of which were common to both categories

(irrelevant features: A3, A4, B3, and B4; see Figure 6-2a, bottom row), and two that were unique to each category (relevant features: A1, A2, B1, and B2; see Figure 6-2a, top row). Individual features could appear in any corner of the exemplar stimulus, meaning there was 24 different orientation combinations for Category A and Category B stimuli (48 exemplars in total). Exemplars were presented in the middle of the rectangular hole against a black background. Each of the four features were 20 mm^2 and were placed in a square formation 55 mm apart from centre to centre. A white line joined the four features together. When the choice keys were presented, these keys appeared 50 mm to the right and left of the white lines, and the choice keys measured 22(w) x 60(h) mm. One of the choice keys was a geometric pattern and the other was a zebra-stripe pattern (see Figure 6-2b).

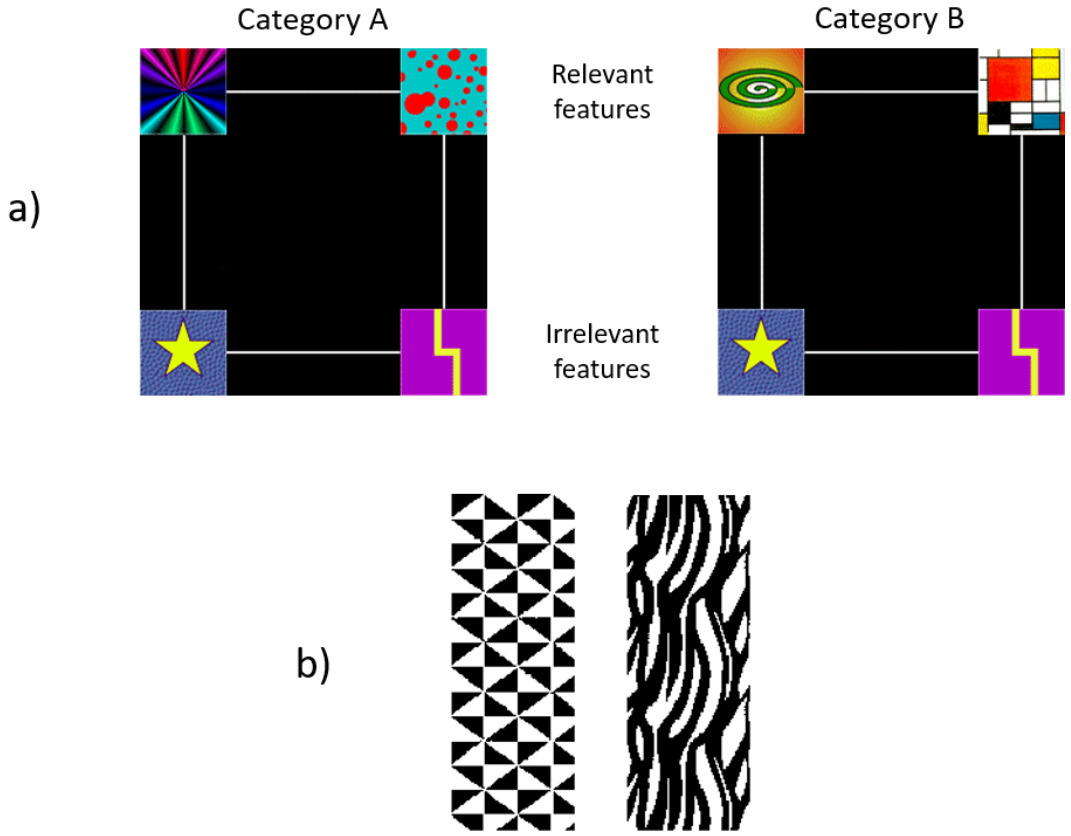

Figure 6-2: The stimuli used in the experiment.

Exemplar stimuli (a) from both categories were made up of four features, two irrelevant and two relevant. For Category A, the relevant features were the rainbow prism (A1) and the polka dots (A2), and for Category B these features were the spiral (B1) and the geometric patchwork (B2). Irrelevant features were the star (A3 and B3) and the lightning bolt (A4 and B4). Individual features could appear in any corner of the exemplar stimulus. Two comparison stimuli (b), the geometric and zebra patterns, were mapped onto the two categories.

6.2.3. Behavioural task

The birds were trained on a simultaneous matching-to-category task. Half of the birds were trained to peck the geometric pattern when a Category A exemplar appeared and the

zebra pattern when a Category B exemplar appeared (A-Geo group; X3, X4, X33, and X36), while the other half were trained with the opposite category-pattern pairings (A-Zebra group; X7, X12, X13, and D16).

Figure 6-3 shows the sequence of events of a trial for an A-Geo bird. A trial began with a 10 s intertrial interval (ITI), in which nothing appeared on the screen. At the end of the ITI, the ready period began, in which a small white dot appeared in the middle of the Perspex panel. The birds were required to peck the dot three times to initiate the rest of the trial. That is, birds essentially chose when a trial would begin. After three pecks to the ready stimulus, a pause period occurred in which nothing appeared on screen, and birds had to inhibit responding for 2000 ms. If the birds made any pecks before the end of the 2000 ms, the pause period was reset. After a full 2000 ms pause, one of the 48 exemplars appeared on screen. On half of the trials, a Category A exemplar would appear, and on the other half of trials a Category B exemplar would appear. Birds were required to peck five times within the bounds of any of the four features. When five pecks occurred, the choice keys appeared on either side of the exemplar, one geometric and one zebra pattern. The position of the patterns (left or right of the exemplar) was counterbalanced across all sessions, so that they appeared on both sides equally on average per session. Birds had to peck the choice key that represented the current exemplar's category to be given 3000 ms access to wheat reward via an illuminated hopper. If birds pecked the wrong choice key, a 3000 ms timeout period signalled by a 50 Hz tone was initiated in which no reward was given, followed by the ITI of the next trial.

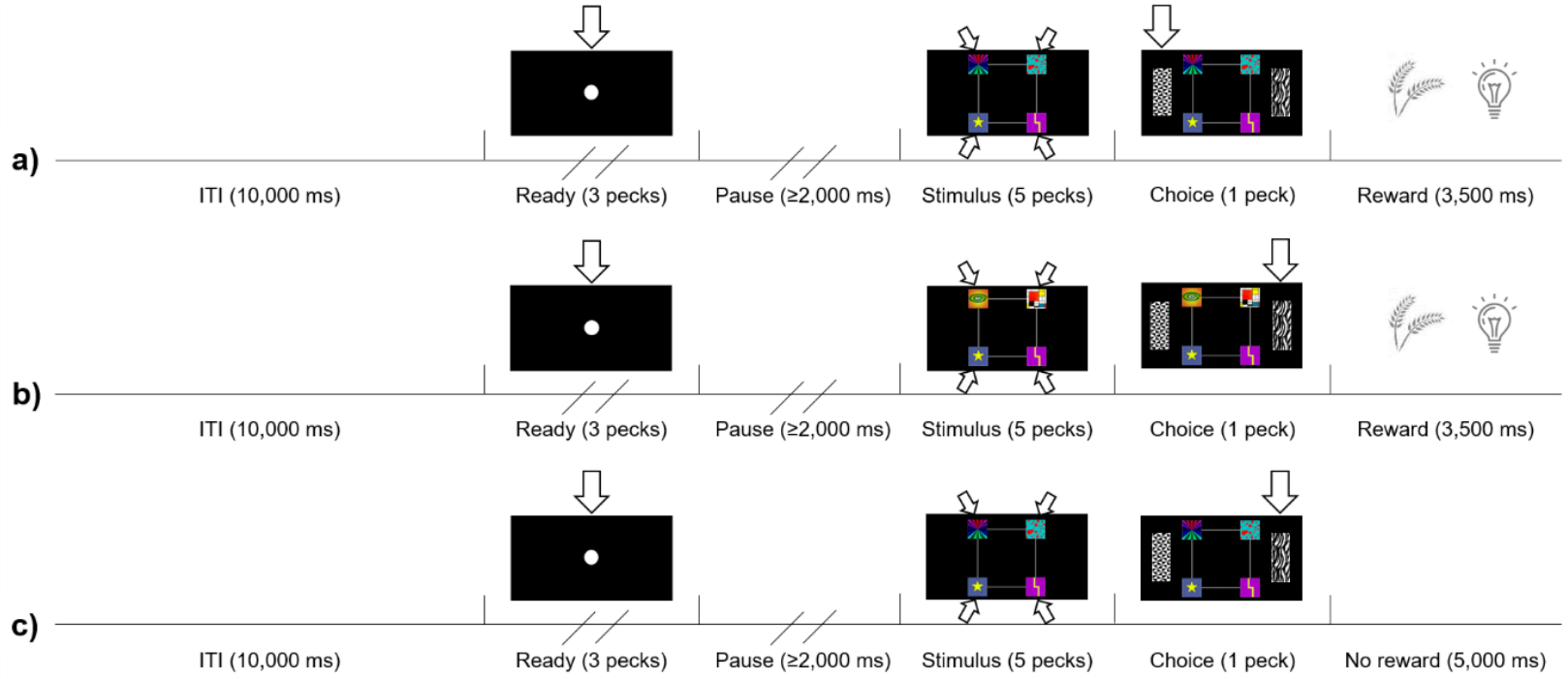

Figure 6-3: Behavioural task.

The sequence of events of Category A and B trials for an A-Geo bird. Trials began with a 10 s ITI, in which nothing was presented on-screen. At the end of the ITI, a ready stimulus (dot) appeared. Birds had to peck the ready stimulus three times to initiate the pause period, in which birds had to refrain from pecking for 2 s. Any pecks during this period reset the timer. After birds had refrained from pecking for 2 s, one of the 48 exemplar stimuli was presented. Birds could peck any of the four features in an exemplar, and after five pecks the choice keys were presented. For A-Geo birds, when the exemplar was from Category A (a), correct pecks to the geometric choice key resulted in 3 s access food reward. When the exemplar was from Category B (b), correct pecks to the zebra choice key resulted in food reward. If the bird pecked the wrong choice key (c), a 3 s time-out period signalled via a 50 Hz tone was initiated, before the next ITI began. A-Zebra birds had to peck the opposite choice keys for Category A and B. ITI; intertrial interval.

6.2.4. Surgery

The birds underwent surgery once they performed at 85% or above for Category A and for Category B separately, for two consecutive days. Microdrives were installed that housed the electrodes used for neuronal recording. Four pigeons had electrodes inserted into the NCL, and four had electrodes inserted into the ENTO. For both regions, two birds had learned the A-Zebra category-pattern pairing and had microdrives installed the left (X7 and X13) and right (X12 and D16) hemisphere, and two birds had learned the A-Geo category-pattern pairing and had microdrives installed in the left (X3 and X4) and right (X33 and X36) hemisphere.

6.3. Results

6.3.1. Histology

All electrode tracks were within the targeted regions as defined by Karten and Hodos (1967). Figure 6-4 shows the reconstructed tracks for all eight birds across both NCL and ENTO.

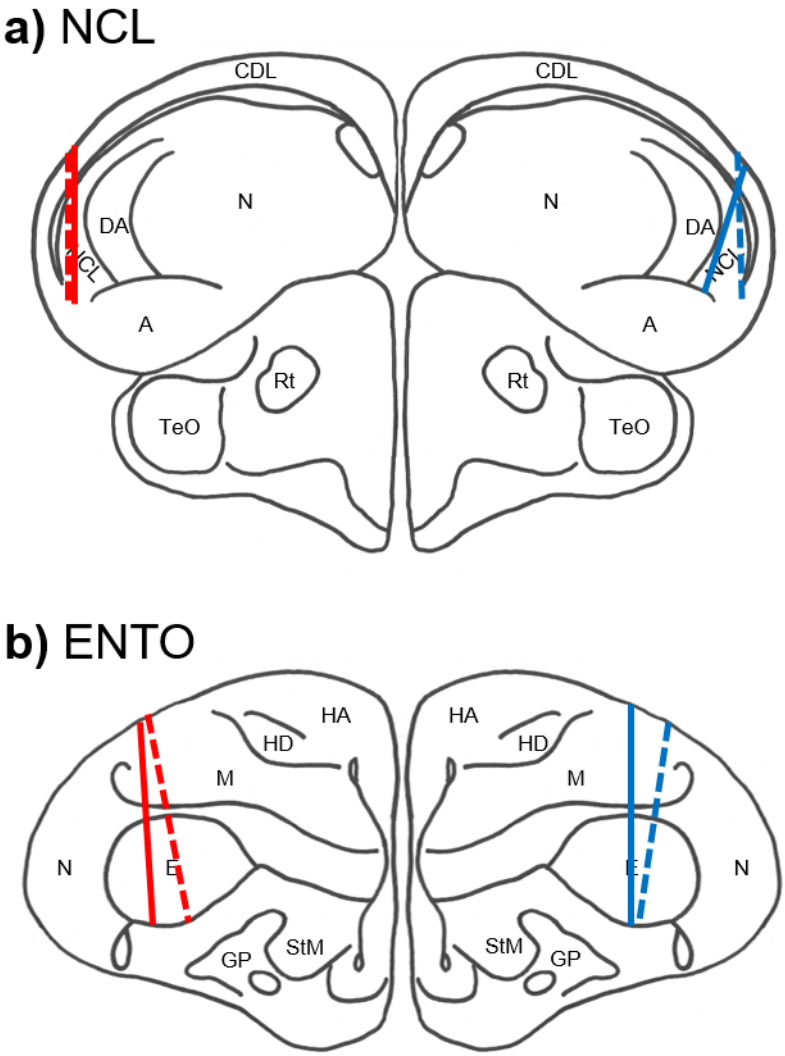

Figure 6-4: The electrode track reconstruction.

(A) NCL. Solid red line – X3; dashed red line – X7; solid blue line – X33; dashed blue line – X12. (B) ENTO. Solid red line – X4; dashed red line – X13; solid blue line – X36; dashed blue line – D16. Brain regions (as defined by Reiner et al., 2004): A, arcopallium; CDL, area corticoidea dorsolateralis; DA, tractus dorso-arcopallialis; E, entopallium; HA, hyperpallium apicale; HD, hyperpallium densocellulare; GP, globus pallidus; M, mesopallium; N, nidopallium; NCL, nidopallium caudolaterale; Rt, nucleus rotundus; StM, striatum mediale; TeO, tectum opticum.

For NCL, the intended track positions were AP +5.5 and ML ±7.5. The track position for one of the two left hemisphere birds (X3) was at AP +5.5, ML +8.25, differing from

the ML by 0.75mm. The track position for the other left hemisphere bird (X7) was at AP +4.75, ML +8.0, differing from the AP by 0.75mm and the ML by 0.5mm. The track position for one of the right hemisphere birds (X12) was at AP +5.0, ML -8.25, differing from the AP by 0.5mm and the ML by 0.75mm. The track position for the other right hemisphere bird (X33) was AP +5.5, ML -8.5, differing only from the ML by 1.0mm.

For ENTO, the intended track positions were AP +9.5 and ML ± 6.0. The track position for one of the two left hemisphere birds (X4) was at AP +9.0, ML +6.25, differing from the intended AP by 0.5mm and the ML by 0.25mm. The track position for the other left hemisphere bird (X13) was as intended at AP +9.5, ML +6.0. The track position for one of the right hemisphere birds (D16) was at AP +9.25, ML -7.0, differing from the AP by 0.25mm and the ML by 1.0mm. The track position for the other right hemisphere bird (X36) was as intended at AP +9.5, ML -6.0.

6.3.2. Behavioural performance

The pigeons took an average of 21.0 (*SD* = 15.5), 24.1 (*SD* = 17.9), 31.1 (*SD*= 25.8), and 34.3 (*SD* = 25.1) days to reach an accuracy of 65%, 75%, 85%, and 85% on two consecutive days, respectively. The quickest bird to reach 85% accuracy was X13 with just six days of training, while the bird that took the longest to reach 85% accuracy was X4 with 85 days of training. Interestingly, the average time taken for birds to learn the task was closer to the Variable Irrelevant group from Castro and Wasserman (2014), rather than the Fixed Irrelevant group which the current behavioural task is based on.

On each training session, we recorded the number of pecks birds made to each of the four stimuli in Category A and B exemplar, as well as their accuracy in correctly categorising A and B exemplars (see Figure 6-5). As each bird took a different number of sessions to reach 85% accuracy, similar to Castro and Wasserman (2014), we created

Vincentized learning curves by grouping all of the training sessions into six blocks (as six was the smallest number of sessions required to reach 85% accuracy; see Kling & Riggs, 1971).

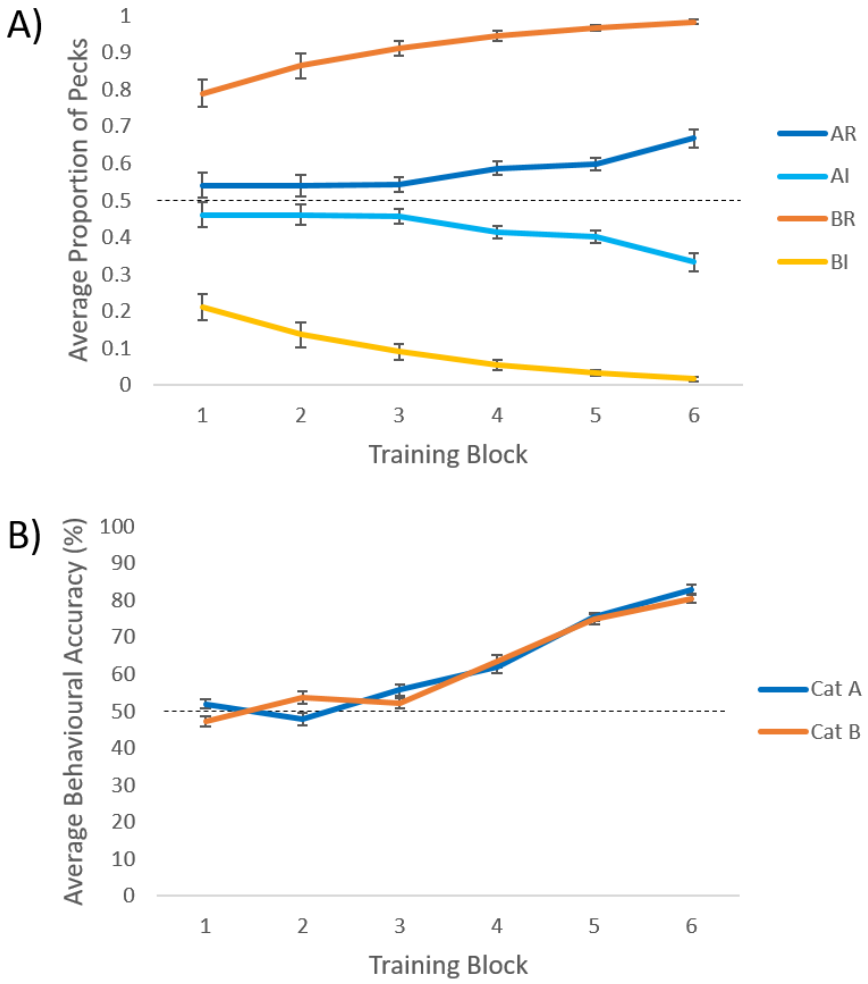

Figure 6-5: Proportion of pecks made and accuracy during training.

A) The average proportion of pecks made to relevant and irrelevant stimuli in both categories during training, across all eight birds. AI: A-irrelevant; AR: A-relevant; BI: B-irrelevant; BR: B-relevant. The dashed line (0.5) represents an equal number of pecks to both irrelevant and relevant stimuli within each category. B) The average behavioural accuracy on both Category A and Category B trials, across all eight birds. The dashed line (50%) represents chance performance. All error bars are ±1 SEM.

Interestingly, at the start of training, birds made an average of 79.0% of their pecks to B-relevant stimuli, while only 21.0% to B-irrelevant stimuli. On the other hand, for Category A stimuli, birds pecked both relevant and irrelevant stimuli fairly equally (54.0% and 46.0%, respectively). For Category B exemplars, pecks to B-relevant stimuli greatly increased (98.4%) and pecks to B-irrelevant stimuli greatly decreased (1.6%) as training progressed. However, for Category A exemplars, pecks to A-relevant stimuli only slightly increased (66.7%) and pecks to A-irrelevant stimuli only slightly decreased (33.3%) as training progressed. Regardless of pecks to A-relevant and irrelevant stimuli not diverging to the same extent as B stimuli, the divergence of A stimuli was still above chance pecking in block six, $t(37) = 5.63$, $p < 0.001$. Furthermore, there was no difference in behavioural accuracy between Category A and Category B trials in block six, $t(52) = 1.68$, $p = 0.1$. Differences in pecking between Category A and Category B exemplars will be discussed in section 6.4.3.

The behavioural performance across all testing sessions for all eight birds, depending on the region they were implanted in, is shown in Figure 6-6.

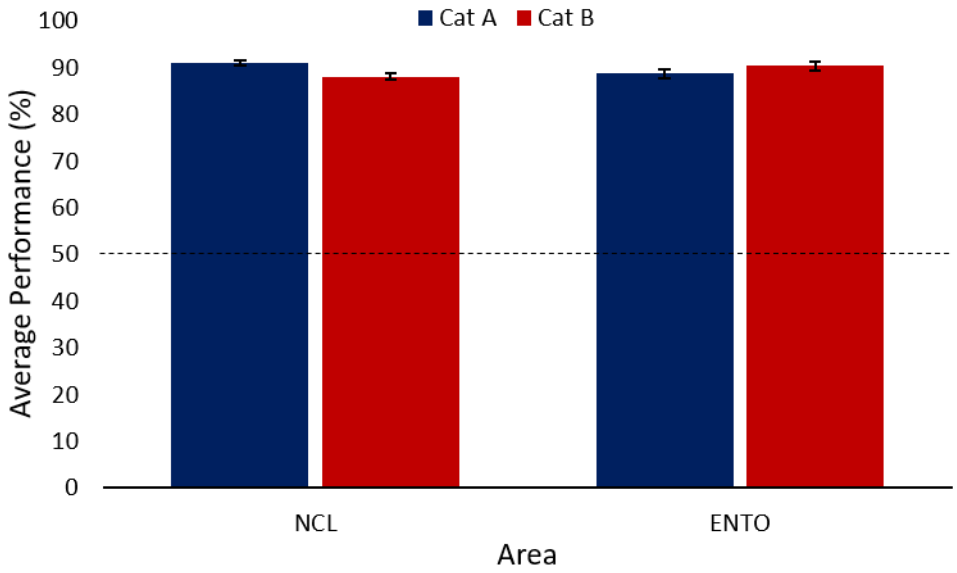

Figure 6-6: Behavioural performance during testing.

The overall behavioural performance for both NCL and ENTO birds on the matching-to-category task. The dashed line represents chance performance (50%). Error bars are ±1 SEM.

All birds performed significantly above chance levels. To see if there was any difference in performance between NCL and ENTO birds, were used a two-way ANOVA with area (2: NCL and ENTO) and category (2: A and B) as factors. There was no significant main effect of category or area, both Fs < 3.27, both ps > 0.072. However, there was a significant interaction effect between category and area, $F(1,208) = 19.19$, $p < 0.001$. To further understand this interaction, we used paired t-tests between the performance on Category A and B trials for both NCL and ENTO birds. There was no difference in performance for the two categories for ENTO birds, $t(97) = 1.57$, $p = 0.12$, but for NCL birds, performance was significantly better during Category A trials over Category B trials, $t(111) = 5.14$, $p < 0.001$. While we are not sure why

NCL birds perform better on Category A over Category B trials, importantly, all birds performed significantly above chance regardless of area or category, $t(419) = 100.18$, $p < 0.001$.

6.3.3. Neural filtering for visually responsive neurons

Neurons were filtered based on whether they were visually responsive or not, on three different levels of analysis (category, relevance, and stimulus). At the overall category level, a neuron was determined as being visually responsive by initially running the two-way repeated-measures ANOVA outlined in Chapter 2. At the relevance level, we grouped the four stimuli that made up the exemplars into pairs based on whether they were relevant or irrelevant features. For example, an A-relevant trial was any trial in which the bird pecked one of the A-relevant stimuli within the exemplar at least three out of the five required times. We then ran the same ANOVA as at the category level except using relevance (A-relevant, A-irrelevant, B-relevant, and B-irrelevant) instead of category as a factor. At the stimulus level, we separated trials based on each of the four stimuli within Category A and Category B exemplars. For example, an A1 trial was any trial in which the bird pecked the A1 stimulus (rainbow) at least three out of the five required times. Again, we ran the same ANOVA as at the category level except using stimulus (1, 2, 3, and 4) instead of category as a factor, for both A and B exemplars. At all three levels of analysis, if there were less than 10 trials for any group (e.g. Category A, A-relevant, A1 stimulus, etc.), the neuron was not used for analysis. For each neuron, we then split the sessions' data into the different trial types depending on whether we were analysing at the category, relevance, or stimulus level (two, four, and two sets of four trial types, respectively) and averaged the neural activity within each of these trial types.

6.3.4. Data analysis

After filtering for visually responsive neurons at the category level, a total of 114 neurons were used for data analysis, 53 from NCL and 61 from ENTO. Of the 53 visually responsive NCL neurons, 37 neurons (69.8%) were classified as inhibitory while the remaining 16 neurons (30.2%) were excitatory. Of the 61 ENTO neurons, 44 (72.1%) were classified as excitatory and the remaining 17 neurons (27.9%) were inhibitory.

After filtering for visually responsive neurons at the relevance level, a total of 33 neurons were used for data analysis, 22 from NCL and 11 from ENTO. Of the 22 visually responsive NCL neurons, 20 (90.9%) were classified as inhibitory, while the remaining 2 neurons (9.1%) were excitatory. Of the 11 ENTO neurons, 6 (54.5%) were classified as excitatory while the remaining 5 (45.5%) were inhibitory. Due to the low numbers of excitatory neurons in NCL, and both types in ENTO, subsequent analyses were restricted to just NCL inhibitory neurons.

After filtering for visually responsive neurons at the stimulus level, a total of 26 neurons were used for data analysis, 23 from NCL and 3 from ENTO. All 23 of the NCL neurons were visually responsive to at least one of the four Category A stimuli, and 20 (87.0%) were classified as inhibitory while the remaining 3 (13.0%) were excitatory. No NCL neurons were visually responsive to any of the four Category B stimuli. Of the 3 visually responsive ENTO neurons, 2 were visually responsive to at least one of the Category A stimuli in an excitatory manner, and 1 was visually responsive to at least one of the Category B stimuli in an excitatory manner. Due to the low numbers of visually responsive neurons in ENTO, and NCL neurons that were excitatory to Category A, we restricted subsequent analyses to just NCL neurons that were inhibitory to at least one of the Category A stimuli.

6.3.5. Population profiles

We constructed population profiles of visually responsive neurons in NCL and ENTO, at the category, relevance, and stimulus levels. At the category level, we averaged across all Category A trials and across all Category B trials for each neuron. At the relevance level, we averaged across all A-relevant, A-irrelevant, B-relevant, and B-irrelevant trials for each neuron. At the stimulus level, we averaged across all trials for individual stimuli within both categories for each neuron. To see if there was any difference in neural activity at the category level, we used a two-way repeated-measures ANOVA with category (2: A vs B) and bin (6: for each of the periods; the middle 300 ms of the ITI, the 300 ms ready period, the middle 300 ms of the pause period, the 300 ms stimulus period, the 300 ms choice period, and the middle 300 ms of the reward period, respectively) as factors, with repeated measures over category and bins (Greenhouse-Geisser corrected). To see if there was any difference in neural activity at the relevance and stimulus levels, we used a two-way repeated-measures ANOVA with relevance or stimulus (4: A-relevant, A-irrelevant, B-relevant, and B-irrelevant; or 4: 1, 2, 3, and 4) and bin (6: the same 6 300 ms periods described above) as factors, with repeated measures over relevance or stimulus and bin (Greenhouse-Geisser corrected). Note that we do not report results for any main effects of bin, as any significant value just reflects variations in the firing rates from one bin to the next, and is generally not of interest, but we do report interaction effects between category/relevance/stimulus and bin.

The population profile for excitatory NCL neurons at the category level (CE) is shown in Figure 6-7a. There was a significant main effect of category in the stimulus period, $F(1,15) = 5.51$, $p = 0.033$, with significantly greater activity on Category B trials than on Category A trials, but not in the other five periods, all Fs < 3.34, all ps > 0.087. There

was no significant interaction effect between category and bin in any of the six periods, all Fs < 1.63, all ps > 0.19.

The population profile for inhibitory NCL neurons at the category level (CI) is shown in Figure 6-7b. There was a significant main effect of category in the ready period, $F(1,36) = 12.44$, $p = 0.001$, and the choice period, $F(1,36) = 8.04$, $p = 0.007$, with significantly more inhibitory activity on Category B trials than on Category A trials in both periods. There was no significant main effect of category in the other four periods, all Fs < 3.63, all ps > 0.065. There was a significant interaction effect between category and bin in the ready period, $F(5,180) = 2.98$, $p = 0.024$, with activity on Category A trials decreasing across the period, but activity on Category B trials remaining relatively stable across the period, but not in the other five periods, all Fs < 1.03, all ps > 0.38.

The population profile for inhibitory NCL neurons at the relevance level (RI) is shown in Figure 6-7c. There was no significant main effect of relevance in any of the six periods, all Fs < 2.69, all ps > 0.065. There was also no significant interaction effect between relevance and bin in any of the six periods, all Fs < 2.03, all ps > 0.073.

The population profile for inhibitory NCL neurons at the stimulus level for Category A stimuli (SI) is shown in Figure 6-7d. There was no significant main effect of stimulus in any of the six periods, all Fs < 1.08, all ps > 0.36. There was also no significant interaction effect between stimulus and bin in any of the six periods, all Fs < 1.55, all ps > 0.16.

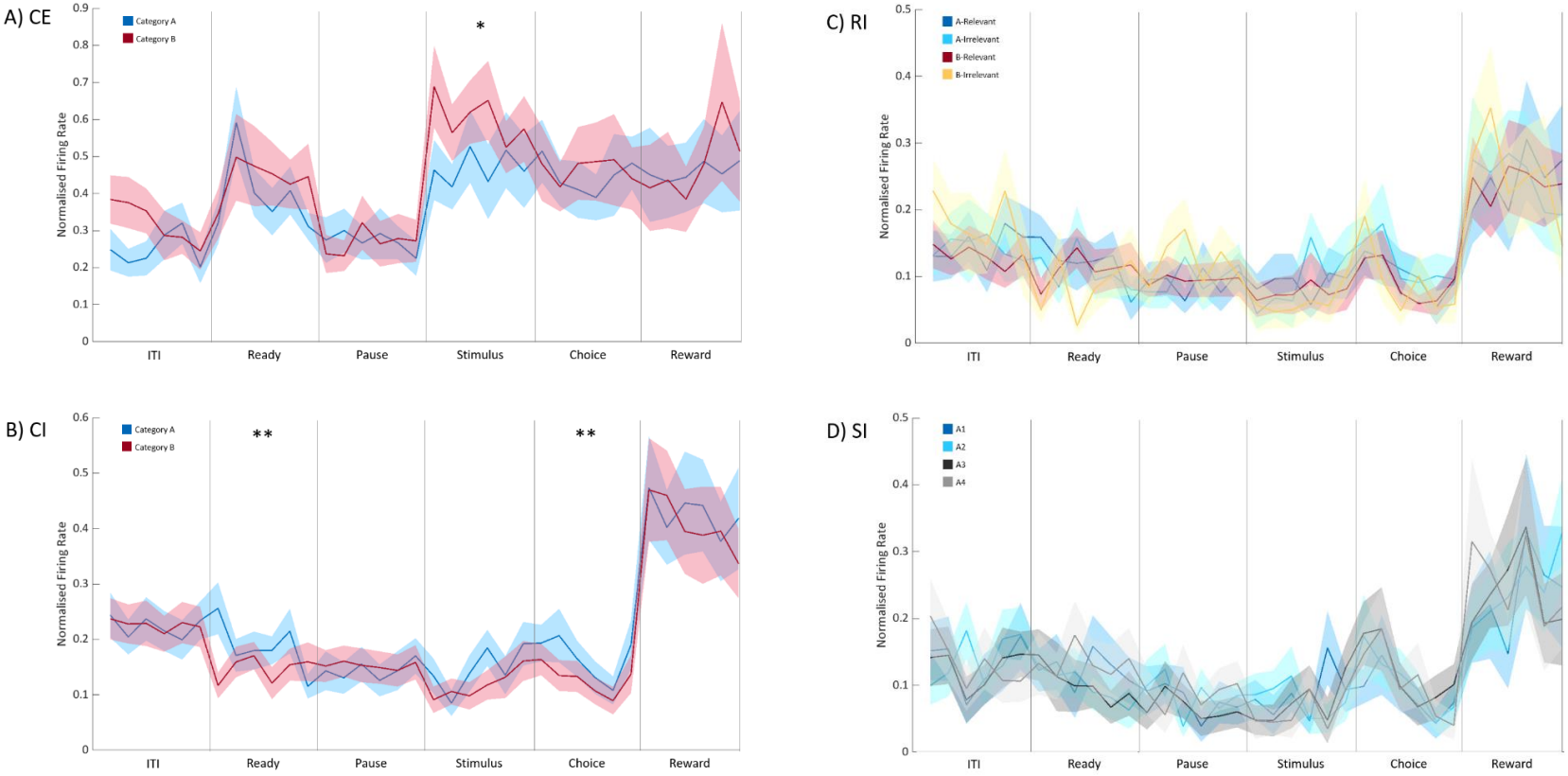

Figure 6-7: The population profiles for NCL neurons.

The population profiles for NCL neurons at the category level (A-B), the relevance level (C), and the stimulus level for Category A stimuli (D). CE: excitatory neurons at the category level; CI: inhibitory neurons at the category level; RI: inhibitory neurons at the relevance level; SI: inhibitory neurons at the stimulus level. Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval. * $p < .05$, ** $p < .01$, *** $p < .001$.

The population profile for excitatory ENTO neurons at the category level is shown in Figure 6-8a. There was no significant main effect of category in any of the six periods, all Fs < 3.93, all ps > 0.054. There was also no significant interaction effect between category and bin in any of the six periods, all Fs < 1.64, all ps > 0.16.

The population profile for inhibitory ENTO neurons at the category level is shown in Figure 6-8b. There was no significant main effect of category in any of the six periods, all Fs < 2.91, all ps > 0.11. There was also no significant interaction effect between category and bin in any of the six periods, all Fs < 2.39, all ps > 0.082.

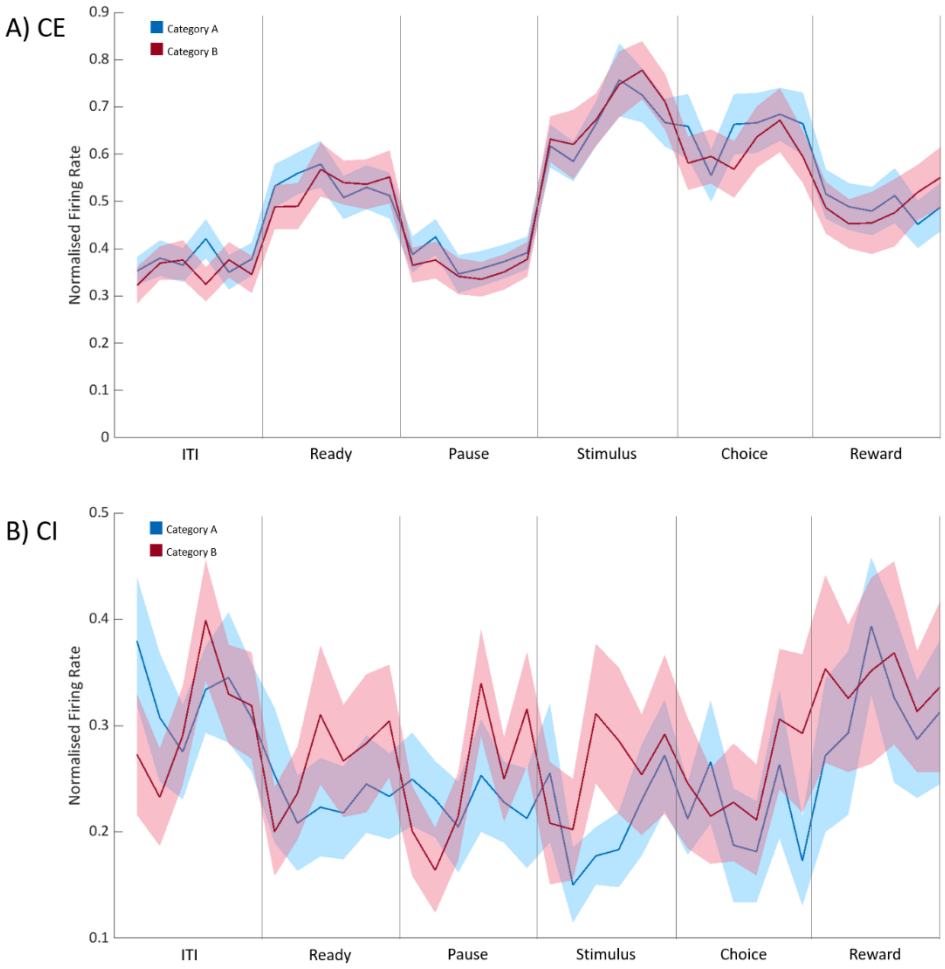

Figure 6-8: The population profiles for ENTO neurons.

The population profiles for ENTO neurons at the category level. CE: excitatory neurons at the category level; CI: inhibitory neurons at the category level. Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval. * $p < .05$, ** $p < .01$, *** $p < .001$.

6.3.6. Categorisation at the single-cell level

One problem with creating population profiles for neurons in the current study is the fact that we only recorded from a small number of neurons, limiting the extent to which we can say that the neural activity found is representative of the population. While we

have a decent number of neurons at the category level, the number of neurons contributing to the population profiles at the relevance and stimulus levels are either very small or non-existent. The small number of neurons is in part attributed to the fact that we only include visually responsive neurons, and at the finer levels like relevance and stimulus, we lack enough trials to properly test for visual responsiveness.

Therefore, we decided to further analyse single neuron activity using data from single trials via Bayesian mixed effects modelling. We decided to fit a Bayesian negative binomial mixed effects model with random intercepts for each neuron at the relevance level (A-relevant vs A-irrelevant vs B-relevant vs B-irrelevant) in order to better understand how neurons responded to features that should predict category membership and features that do not hold information about category membership. We took the average firing rate of neurons on each trial during both the 300 ms stimulus period and the 300 ms choice period, for NCL and ENTO neurons separately. Each model was run with eight chains and 10,000 iterations, and samples were drawn using No-U-turn sampling (NUTS). We were unable to apply Bayesian mixed effects models at the stimulus level due to time restrictions. The posterior distribution slopes for each relevance condition are shown for all four models in Figure 6-9.

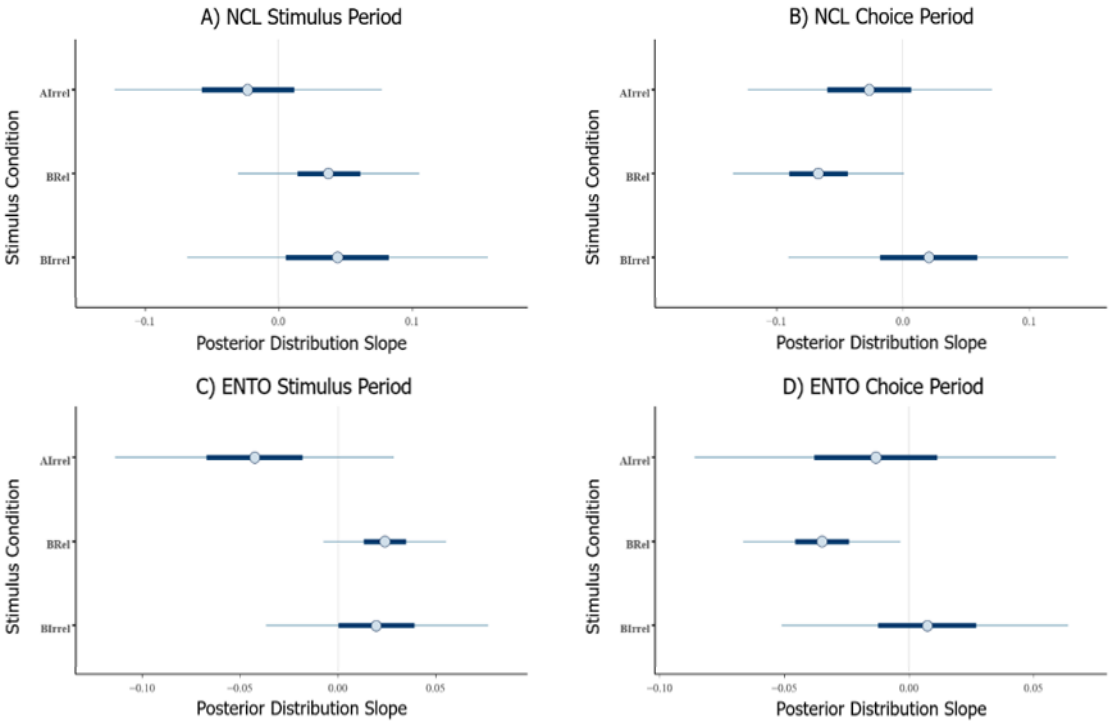

Figure 6-9: Posterior distribution slopes for each stimulus condition.

The posterior distribution slopes of the firing rates for each of the stimulus conditions for: A) NCL neurons in the stimulus period; B) NCL neurons in the choice period; C) ENTO neurons in the stimulus period; and D) ENTO neurons in the choice period. In each plot, the A-relevant condition is used as the reference condition (vertical grey lines), and all other conditions are plotted relative to it. For each condition, the circle represents the estimated mean firing rate, the dark blue line represents 50% of the spread, and the light blue line represents 100% of the spread.

For NCL neurons, in the stimulus period, there was a 98.0% decrease in firing rates from A-relevant trials to A-irrelevant trials, but a 104.1% increase in firing to both B-relevant and B-irrelevant trials. However, none of the conditions were significantly different from each other in the stimulus period. In the choice period, there was a 97.0% decrease in firing rates from A-relevant trials to A-irrelevant trials, and a 102.0% increase in firing rates to B-irrelevant trials, although neither of these were significant.

Interestingly, there was a 93.2% decrease in firing rates from A-relevant trials to B-relevant trials, which was significant.

For ENTO neurons, in the stimulus period, there was a 96.1% decrease in firing rates from A-relevant trials to A-irrelevant trials, but a 102.0% increase in firing rates to both B-relevant and B-irrelevant trials. However, none of the conditions were significantly different from each other in the stimulus period. In the choice period, there was a 99.0% decrease in firing rates from A-relevant trials to A-irrelevant trials, and a 101.0% increase in firing rates to B-irrelevant trials, although neither of these were significant. Interestingly, there was a 97.0% decrease in firing rates from A-relevant trials to B-relevant trials, which was significant.

6.4. Discussion

6.4.1. Summary of findings – category level

We analysed a total of 114 visually responsive neurons at the category level, 53 from NCL and 61 from ENTO. In NCL, the majority of neurons were inhibitory (69.8%), while the majority of neurons in ENTO were excitatory (72.1%). These visually responsive neurons were further analysed using a two-way repeated-measures ANOVA (Greenhouse-Geisser corrected) to see whether neural activity differed between Category A and Category B within each task period. In ENTO, we found no differences in neural activity to the two categories in any period. In NCL, we found that activity on Category B trials was more excitatory in the stimulus period for excitatory neurons than activity to Category A trials, and similarly, activity to Category B trials was more inhibitory for inhibitory neurons than Category A trials in the choice period.

6.4.2. The role of NCL at the category level

We predicted that NCL would encode categorical information beyond the visual properties of the stimuli, i.e. an amalgamation of both behavioural and category-reward associations. That is, we expect NCL neurons to encode differences between categories during the choice and reward periods. We found that inhibitory neurons displayed a significant difference in activity between Category A and B trials during the choice period, indicating that these neurons were encoding differential behavioural responses (choices) associated with each category. That is, when a behavioural decision was required in the choice period, inhibitory neurons associated Category A with one response, and B with the other response. The NCL is known to be involved in decision-making and goal-directed behaviour (Liu, Wan, Li, Shang, & Shi, 2017; Starosta, Güntürkün, and Stüttgen, 2013), and thus the pattern of activity within the choice period for inhibitory neurons appears to demonstrate NCL's involvement in decision making during categorisation. Similarly, differential activity of NCL neurons in the choice period during an abstract rule task has also been found in carrion crows (Veit & Nieder, 2013). Since we counterbalanced groups during training so that half the birds were trained to peck the geometric pattern when it was a Category A exemplar, and half were trained to peck the zebra pattern, we argue that the difference during the choice period is not caused by one choice pattern being more visually stimulating than the other. Furthermore, the difference in activity to Category A and B trials in the choice period cannot have been caused by differential motor responses (i.e., pecking the left or right of the screen) as we also counterbalanced which side each choice key appeared on.

While we did not find similar differential firing to the categories in the choice period of excitatory neurons, we did find a difference in activity during the stimulus period.

Although the NCL is generally associated with reward-related processing and decision making, we do know that the NCL is capable of coding visual differences in stimuli as well (see Johnston et al., 2017b). In primates, the PFC has been shown to be stimulus-selective, although not to the extent of IT cortex (Miller, Erickson, & Desimone, 1996), and also associated with attention and the behavioural relevance of a stimulus (Desimone, 1996; Rainer, Asaad, & Miller, 1998). Therefore, it seems reasonable for the NCL to also be involved in processes beyond simple reward processing.

Furthermore, lesions to ventral PFC causes impairments for visual memory of colours (Passingham, 1975). In our behavioural data, it can be seen that all birds preferred pecking the Category B-relevant stimuli (spiral and geometric patchwork) compared to irrelevant stimuli. On the other hand, for Category A exemplars, birds tended to peck somewhat equally between the relevant stimuli (rainbow prism and polka dots) and the irrelevant stimuli. It may be that for Category A stimuli, birds struggled to differentiate between relevant and irrelevant stimuli as all four are somewhat blue-ish in colour, whereas Category B-relevant stimuli are more yellow and therefore much more distinct from the irrelevant stimuli. We argue that the reason we find differential activity in the stimulus period may be reflective of NCL's involvement in colour discrimination, attention, and behavioural relevance, much like PFC. Thus, at the point where birds are presented with the exemplars (stimulus period) colour differences between the categories may lead to more attention to behaviourally relevant stimuli for Category B exemplars, and less so to Category A exemplars, resulting in differential neural activity.

However, somewhat unexpectedly, the difference in activity to each category did not carry on through the reward period in inhibitory neurons or re-emerge in excitatory neurons. We know that the NCL is highly implicated in reward processing (Dykes et al.,

2018; Johnston et al., 2017a; Lengersdorf et al., 2014), and in particular, shows reward-category associations during categorisation (see Chapter 5; Kirsch et al., 2009). One reason why we may not have found differential activity to the categories during the reward period was a lack of differential reward. The SMC paradigm used in the current study eliminated the differential reward used in the S+/S- paradigm in Chapter 5 by rewarding birds after responses to both categories, instead of just one. The reward given to our birds was also the exact same across both categories. It has been shown that the NCL is able to switch away from reward-related processing when there are no differential rewards (Johnston et al., 2017b), although categorisation studies have shown that the NCL can still associate the same reward differentially to each category (Kirsch et al., 2009). The flexible nature of the NCL may make it difficult to predict whether a paradigm will elicit reward-related processing or not, and may also depend on hemisphere and which section of the NCL is recorded from (i.e. anterior, posterior etc.).

6.4.3. The role of ENTO at the category level

We predicted that ENTO would encode category-specific information based on visual information i.e., the visual properties of the stimuli within a category. That is, we expect ENTO neurons to encode differences between categories during the stimulus period. However, we did not find any differences in activity to Category A and B trials in any period, for both inhibitory and excitatory ENTO neurons. The lack of difference in the stimulus period is especially surprising considering we did find differences in NCL neurons. For NCL neurons, we argue that differences in the stimulus period arise from the degree to which relevant and irrelevant stimuli differ in colour, which then leads to heightened attention to B-relevant stimuli compared to A-relevant stimuli. Thus, as ENTO as a visual area, we would also expect to see this pattern in ENTO neurons.

However, it may be possible that a difference between Category A and B does exist, but has fallen victim to Simpson's paradox; essentially, significant differences do exist within smaller subsets of the data, but when averaged, the differences in the subsets cancel each other out (for a review, see Wang, Wu, Tu, & Feng, 2018). That is, within Category A trials, it might be true that activity to A-relevant and A-irrelevant trials is fairly similar. However, within Category B trials, activity may be significantly greater to B-relevant trials than B-irrelevant trials due to the fact that B-relevant stimuli are so visually distinct from the irrelevant stimuli. Then, because we are averaging across relevant and irrelevant trials at the category level, activity to Category A and B balances out to a similar level to one another. We would also expect that Simpson's paradox is more likely to occur within the population of ENTO neurons than in NCL as a distinction between relevant and irrelevant stimuli is more likely to be visually based in ENTO, whereas in NCL relevancy should be more important in terms of guiding behaviour and decision-making. Therefore, simply looking at neural activity at the category level may not be as informative as examining activity at the relevance level, especially within a visual area like ENTO.

6.4.4. Summary of findings – relevance level

We analysed a total of 33 visually responsive neurons at the relevance level, 22 from NCL and 11 from ENTO. As with neurons at the category level, the majority of NCL neurons were inhibitory (90.9%) while the majority of ENTO neurons were excitatory (54.5%). These visually responsive neurons were analysed further using the same two-way ANOVA applied at the category level, but with relevance (A-relevant, A-irrelevant, B-relevant, and B-irrelevant) as a factor instead of category. While the number of neurons in ENTO were too few to construct population profiles, in NCL, we found no differences between any of the relevance conditions in any task period.

Due to the low numbers of neurons when filtering at the relevance level, we decided to analyse firing rates on individual trials of the stimulus and choice periods using Bayesian mixed effects modelling. In both NCL and ENTO, there were no significant differences in firing rates between any of the four relevance conditions in the stimulus period. However, in the choice period, both NCL and ENTO neurons showed a significant decrease in firing rates during B-relevant trials compared to A-relevant trials.

6.4.5. The role of NCL and ENTO at the relevance level

We were only able to analyse NCL neurons as a population at the relevance level. As NCL is implicated in decision-making, reward, and behavioural relevance, we expect to see differences in activity between relevant and irrelevant trials in the choice and reward periods. However, when looking at NCL neurons as a population, we did not find any differences in activity between any relevance conditions in any period. While we are unsure why there is a lack of differences between the relevance conditions among the population of NCL neurons, it is likely due to the low number of neurons after filtering for visual responsiveness.

We attempted to fix the problems caused by low numbers of visually responsive neurons by examining relevancy at the single trial level across neurons. Using Bayesian mixed effects modelling in the stimulus and choice periods for both NCL and ENTO neurons, we now found a significant difference in firing rates between B-relevant and A-relevant trials. In both NCL and ENTO, firing rates to B-relevant stimuli was significantly lower than firing rates to A-relevant stimuli in the choice period, but not the stimulus period. We argue that the difference in firing rate between A-relevant and B-relevant stimuli is again likely due to colour differences. As stated previously, B-relevant stimuli are both yellow/orange, and are visually distinct from all other stimuli which are more

blue/purple in colour. Thus, in ENTO, we argue that the difference in firing rates to B-relevant stimuli is caused by ENTO neurons encoding differences in the visual properties of the stimuli, although it is unexpected that differences occur in the choice period and not the stimulus period (see below for further discussion). In NCL, we argue that the difference in firing rates to B-relevant stimuli is also caused by differences in the visual properties of the stimuli, but that the colour difference is likely to be associated with behavioural relevance. That is, yellow stimuli (B-relevant) are distinct from blue stimuli (irrelevant), so behavioural choices are easier to make, and is why we only see differences in the choice period rather than the stimulus period.

As mentioned above, we would expect ENTO to encode differences in the visual properties of the stimuli and that differences would emerge in the stimulus period; however, we only find differences in firing rates within the choice period. One possibility is that birds are not attending to relevancy until the choice period when the choice keys appear. As posited by Castro and Wasserman (2014), there are three possible strategies for completing the task: 1) birds could look at the whole exemplar, place pecks on a random feature, and then make their decision; 2) birds could place pecks on a random feature, look at the whole exemplar, and then make their decision; or 3) birds could look at the whole exemplar, place pecks to relevant stimuli, and then make their decision. In their study, Castro and Wasserman (2014) argue that on the basis of their behavioural data, in which relevance of the pecked stimuli was strongly correlated with behavioural performance, the birds were using the third strategy during the task. However, in our study, we found that birds were able to solve the discrimination successfully, regardless of which stimulus they had pecked. Particularly, the proportion of pecks made to relevant and irrelevant stimuli for Category A exemplars did not diverge in the same manner that

Category B stimuli did as training progressed. The fact that our birds could peck an irrelevant stimulus and still choose the correct response suggests that they must be looking at the whole exemplar after placing their pecks. Therefore, it is possible that the fact that we only find differences in firing rates to relevance conditions in the choice period for ENTO neurons is because the birds do not look at the whole exemplar until the choice period has commenced, and visual differences are not able to be encoded until this point.

The next question is why our birds employ a different strategy to the birds from Castro and Wasserman (2014), when both paradigms are nearly the same. In fact, our birds, despite being trained on the FI paradigm, mimic the behavioural results from Castro and Wasserman (2014)'s VI group following the introduction of novel relevant features. When novel relevant features were introduced into the category exemplars, VI-trained pigeons took longer to learn the new discrimination and also reduced their pecks to relevant features. However, once re-learned, behavioural accuracy was no different form the FI group. We are not sure what caused our birds to tend towards a different strategy than birds in Castro and Wasserman (2014).

6.4.6. Summary of findings – stimulus level

We analysed a total of 26 visually responsive neurons at the stimulus level, 23 from NCL and 3 from ENTO. All NCL neurons were inhibitory and all ENTO neurons were excitatory. While there were too few ENTO neurons and no neurons in NCL that were responsive to Category B stimuli to create population profiles, a population profile for all 23 NCL neurons that were visually responsive to Category A stimuli was analysed using the same ANOVA as at the relevance level, but with stimulus as a factor instead of relevance. We found no differences between any of the four Category A stimuli in any

task period. We were unable to further analyse firing rates using Bayesian modelling at the stimulus level due to time restrictions.

6.4.7. The role of NCL at the stimulus level

We did not expect that NCL neurons would show any differences in activity to each of the four individual Category A stimuli for three reasons. First, in our population profiles at the relevance level, we did not find any differences in the relevance conditions in NCL. Secondly, we would likely not have a suitable number of neurons for analysis, which was indeed the case. Thirdly, at the stimulus level, it is more likely that individual stimuli will be coded differently based on the differences in their visual properties, rather than any behavioural meaning, and so we would expect ENTO neurons to be more involved at this level than NCL neurons. Therefore, even with such low numbers of neurons contributing to the population profile, it is not surprising that NCL neurons did not differentiate between the four stimuli in any of the periods.

6.4.8. Limitations of the study

We decided to employ the SMC paradigm in the current study to rectify limitations with our S+/S- paradigm from Chapter 5. In the S+/S- paradigm, the fact that differential reward existed made it difficult to parse whether differences in activity represented categorical encoding, or simply the anticipation of reward. While the SMC paradigm eliminated potential confounds caused by differential reward, the current study is not without its own limitations as well. First, the distinctiveness of the relevant stimuli differs between categories. As previously mentioned, B-relevant stimuli were much more distinct from irrelevant stimuli, while A-relevant stimuli were not so distinct. While it does not seem to impact on pigeons' accuracy, the highly distinct B-relevant stimuli

appear to attract much more attention (measured by the rate of pecking to B-relevant stimuli) than A-relevant stimuli.

Along the same vein, it is difficult for us to determine how attention is distributed among stimuli that are less distinct form one another, as peck tracking in these cases is not the best measure of attention. We define a trial as being A-relevant or A-irrelevant when at least 3 out of the 5 required pecks are given to a particular stimulus as peck location is purported to be a good measure of attention. However, we know that our birds must be employing a strategy in which pecks are distributed randomly and then the whole exemplar is assessed, which means that we are unable to determine which features their attention is directed to.

Thirdly, the SMC paradigm could be improved by the addition of a delay period between exemplar presentation and the choice period. A delay would help distance the choice period temporally from the stimulus period, and likely encourage the pigeons to look at the whole exemplar before choosing where to peck (as the exemplar would disappear after 5 pecks, rather than remaining on screen). Therefore, the current strategy employed by the pigeons would not be viable, and they would be forced to attend to features that were relevant during the stimulus period.

CHAPTER 7 | DELAYED MATCHING TO THE CATEGORIES OF PICASSO AND MONET PAINTINGS

In this Chapter, the final of four categorisation studies will be presented, in which birds are trained to discriminate between a subset of the same Monet and Picasso paintings used in Chapter 5. In the previous study, we used a simultaneous matching-to-category (SMC) paradigm, which managed to rectify limitations with the semi-symbolic and S+/S- paradigms (Chapters 4 and 5), and also allowed us to uncover how neurons in NCL and ENTO are involved in categorisation at both the single and population level. However, one challenge with the previous study was that the birds found it difficult to learn the task (as reflected in the length of training required to pass criterion compared to our previous studies), which is likely due to the complex stimuli used in the experiment. Thus, we decided to use a subset of the Monet and Picasso stimuli used in Chapter 5, as birds learned to discriminate these categories more quickly and easily. We also decided to add a delay period between the stimulus and choice periods which was absent in the previous study. For the current study, we used single-unit electrophysiology to record neural activity in NCL, ENTO, and MVL during the Picasso/Monet delayed matching-to-category (DMC) task.

It should be noted that due to time restrictions with the thesis the results presented in this Chapter are from only one bird and should be considered as preliminary in nature.

7.1. Introduction

7.1.1. The delayed matching-to-category (DMC) paradigm

In primates, the delayed matching-to-category (DMC) paradigm has been used in several studies to understand how categorisation is represented neurally. Similar to the DMS paradigm described in section 3.1.3., the DMC task includes a delay period between presentation and comparison periods, but rather than choosing the same stimulus seen, animals are required to choose the category a stimulus belongs to.

Freedman et al. (2001; 2003) created morphed prototype images of cats and dogs and presented them to rhesus monkeys who had never been exposed to these animals before. The morphs ranged from being completely unambiguous (100% cat or dog), to being mostly comprised of one category (80% cat and 20% dog, or vice versa), to mostly ambiguous (60% cat and 40% dog, or vice versa). Morph images were categorised based on what category made up the majority of the image, that is, 80:20 and 60:40 cat/dog morphs were classed as cats and vice versa for dogs (see Figure 7-1). Monkeys were presented with one of these morph images followed by a 1000 ms delay period. Following the delay, a prototype from one of the categories was presented. If the prototype was from the same category as the previously seen morph image, the monkeys had to release a lever to receive reward. If the prototype was from the other category, then monkeys had to keep pressing the lever until a prototype from the same category appeared. Freedman et al. (2001; 2003) found that the monkeys were able to correctly categorise morph images at least 90% of the time, even when the morphs were ambiguous.

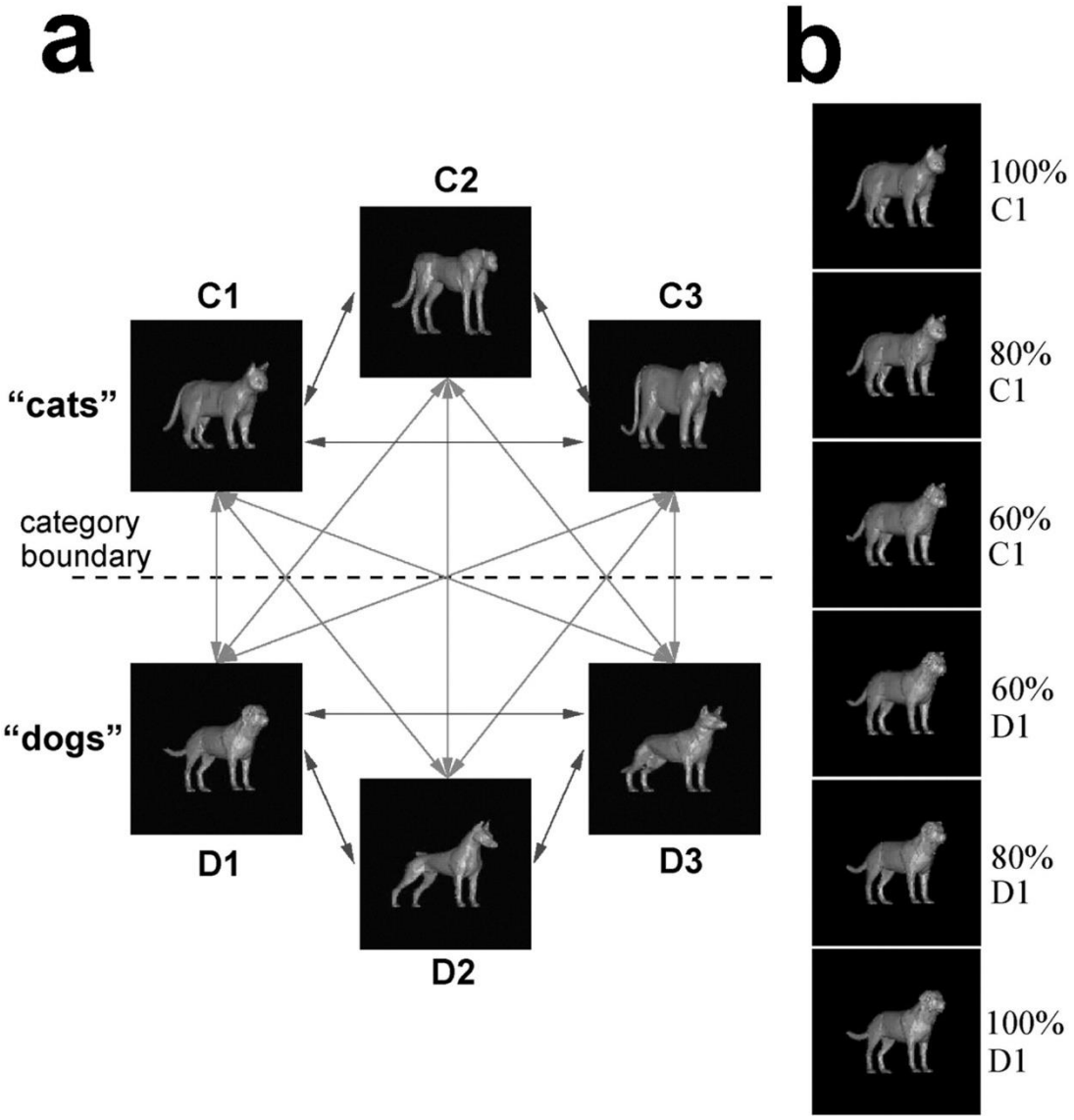

Figure 7-1: Examples of the cat/dog morph images used in Freedman et al. (2003).

(A) The three prototype cat images and three prototype dog images. In total, 54 morphed images were created between the six prototypes. (B) Examples of the morph images created from prototype C1 and D1. The first three morphs belong to the 'cat' category, while the last three morphs belong to the 'dog' category. Adapted from Freedman et al. (2003).[7]

[7] Freedman, D. J., Riesenhuber, M., Poggio, T., & Miller, E. K. (2003). A comparison of primate prefrontal and inferior temporal cortices during visual categorisation. Journal of Neuroscience, 23(12), 5235-5246. https://doi.org/10.1523/JNEUROSCI.23-12-05235.2003. Copyright 2003 Society for Neuroscience.

Freedman et al. (2001; 2003) recorded neural activity in primate IT cortex and PFC during the morph DMC task. Neurons were classified as being category sensitive if there was a difference in activity between the two categories, that is, if activity to all cat images was different to activity to all dog images. During the sample period, Freedman et al. (2003) found that more neurons in IT cortex were category sensitive (27%) compared to PFC neurons (17%). Freedman et al. (2003) argues that the fact that more IT cortex neurons were category-sensitive in the sample period when the stimuli are presented indicates that IT cortex neurons are likely encoding the differences in visual properties of categories. Conversely, during the delay period, more PFC neurons were category sensitive (18%) than in IT cortex (9%), suggesting that PFC activity is more likely to reflect information about category membership beyond visual properties, and can hold this information in working memory. While both PFC and IT cortex neurons displayed differences in activity between categories, both areas also showed a lack of difference between individual stimuli within each category, indicating that neurons were classifying all cat images as cats, and all dog images as dogs.

7.1.2. Aims and objectives of the study

The aim of the current study was to investigate how the categories of 'Picasso' and 'Monet' are represented in NCL, ENTO, and MVL, and the involvement of these areas in the categorisation process. Like our other categorisation studies, we expect that NCL will encode categorical information beyond the visual properties of the stimuli, i.e. an amalgamation of both behavioural and category-reward associations. On the other hand, we expect that ENTO will encode category-specific information based on visual information i.e., the visual properties of the stimuli within a category, and MVL will encode categorical information in a similar yet more complex manner than in ENTO. In

the current study we will also explore how category information will be represented neurally when a delay between stimulus presentation and the choice period is added by implementing a modified version of the DMC paradigm. However, our version of the DMC paradigm will present options from both categories during the comparison period, in order to ensure that birds will execute the same motor response regardless of whether their choice is correct or incorrect. Based on the findings of Freedman et al. (2001; 2003) who employ a similar paradigm, we also expect that we will find more category-sensitive neurons during the delay period in NCL, while in MVL and ENTO we expect to find more category-sensitive neurons during the stimulus period.

7.2. Materials and Methods

7.2.1. Subjects

The subject (D4) was one experimentally naïve pigeon (*Columba livia*).

7.2.2. Apparatus and stimuli

The Perspex panel that was situated in front of the monitor had six square holes measuring 60 x 60 mm, arranged in a two (row) by three (column) grid. The holes were 65 mm apart, from centre to centre. We used a selection of the 7 Monet and 7 Picasso stimuli outlined in Chapter 5, which were chosen from the list of paintings in Watanabe et al. (1995). Sample stimuli were presented in the top middle square and comparison stimuli were presented on the outer top squares and appeared against the black background of the monitor. There was a total of eight different stimuli, four of which were Monet paintings, and four which were Picasso paintings (Monet: '*Garden at Sainte-Adresse'*, '*Camille Monet and a Child in the Artist's Garden in Argenteuil*', '*Field of*

Poppies, '*Still Life with Pears and Grapes*'; Picasso: '*Guitar, Bottle and Fruit*', '*Artist and his Model*', '*Jug, Candle and Enamel Pan*', '*The Women of Algiers*'). As in the study outlined in Chapter 5, the paintings were in black and white, and had been cropped from one corner so as to best preserve the essence of the original painting. Images were then resized to 100 x 100 pixels.

7.2.3. Behavioural task

The bird was trained on a delayed matching-to-category (DMC) task. The set of Picasso paintings served as one category, while the set of Monet paintings served as the second category. Figure 7-2 shows the sequence of events during the DMC task. Trials began with an intertrial interval (ITI), in which nothing was presented on screen for 10 s. At the end of the ITI, one of the four Monet and Picasso paintings was presented on-screen (sample period). The bird was required to peck the stimulus two times, which removed the stimulus from the screen and initiated the delay. After a delay of 3000 ms, a different painting from the same category as the stimulus shown in the sample period and a painting from the other category were presented (comparison period). Pecks to the comparison stimulus that came from the same category as the sample stimulus resulted in 2000 ms access to wheat reward via an illuminated hopper. Pecks to the wrong category resulted in a 2000 ms time-out period, in which a blank screen coupled with a 500 Hz tone was presented, followed by the ITI of the next trial. A correction routine was in place for both training and testing, such that all incorrect trials were repeated until the correct response was made. Only the first attempt at each trial was calculated for behavioural performance. Within a session, each of the stimuli were randomly presented 7 times each, resulting in a total of 56 trials per session.

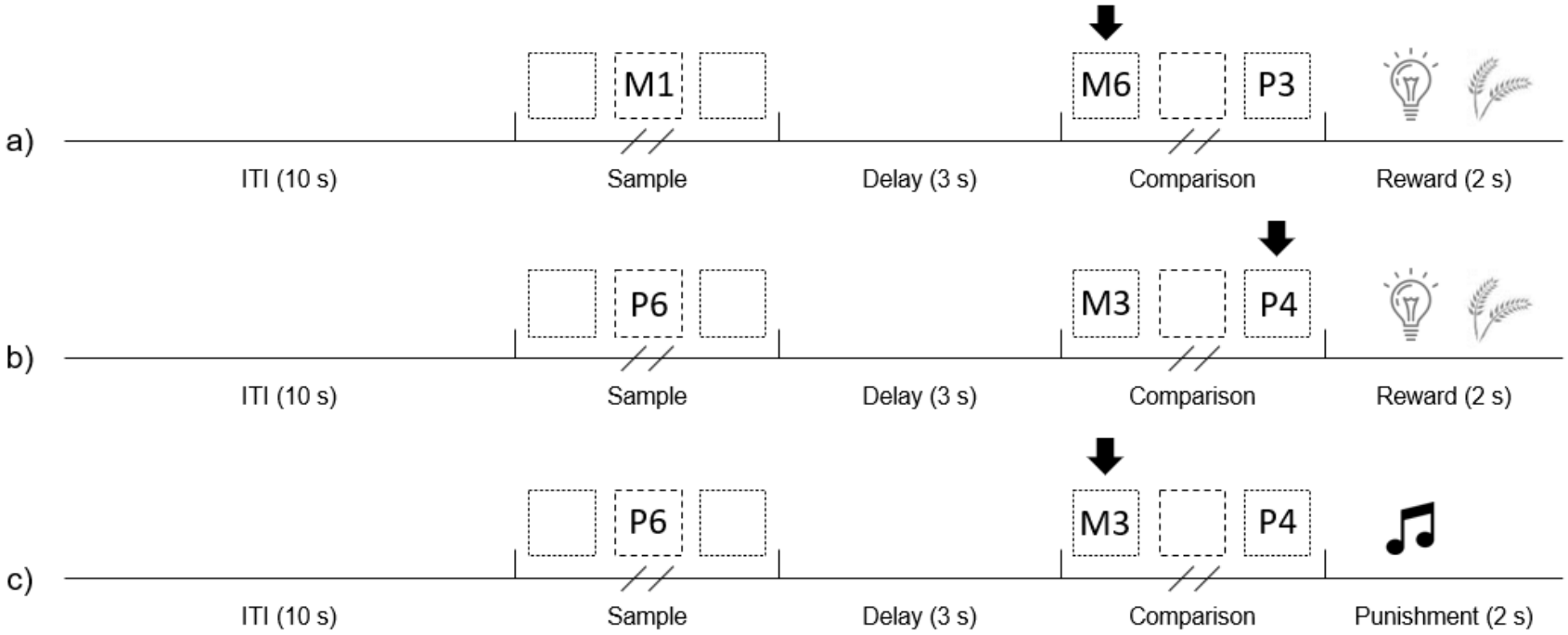

Figure 7-2: The delayed matching-to-category task.

The sequence of events on the delayed matching-to-category (DMC) task. When a Monet painting served as the sample stimulus (a), birds were required to peck the other Monet painting during the comparison period to obtain a wheat reward. When a Picasso painting served as the sample stimulus (b), birds were required to peck the other Picasso painting to obtain reward. If the opposite category to the previously presented stimulus was chosen (c), for example, choosing the Monet category when the sample stimulus had been a Picasso painting, the birds were not rewarded, and a time-out punishment period ensued. ITI, intertrial interval.

7.2.4. Surgery

The bird underwent microdrive surgery after it had reached at least 70% performance to both Monet and Picasso stimuli. The bird initially had electrodes inserted into the right NCL, before undergoing a second surgery to insert a second set of electrodes into the right MVL and ENTO. For MVL, the electrodes were positioned at AP +9.5, ML -6.0, DV = 1.0. When the electrodes reached the end of MVL, the electrodes were advanced a further 960 μm to the start of ENTO at AP +9.5, ML -6.0, DV = 3.0.

7.3. Results

7.3.1. Histology

All electrode tracks were within the targeted regions as defined by Karten and Hodos (1967). Figure 7-3 shows the reconstructed tracks for the one bird (D4) across all three regions.

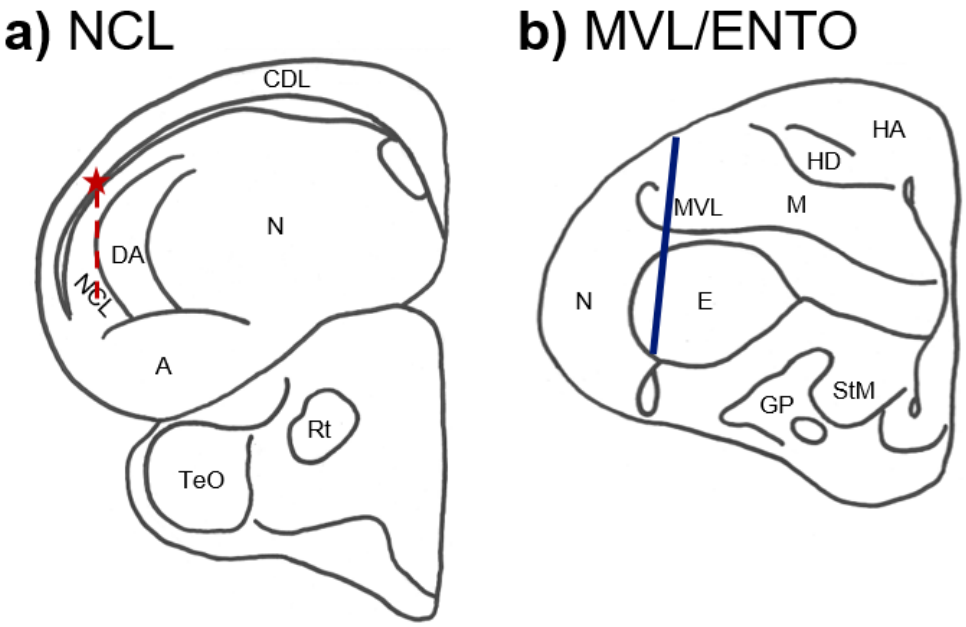

Figure 7-3: The electrode track reconstruction.

The electrode track reconstruction for D4 in A) NCL; and B) MVL and ENTO. In NCL, the red star represents the recovered entry point, and the dashed line denotes the probably location of the track. Brain regions (as defined by Reiner et al., 2004): A, arcopallium; CDL, area corticoidea dorsolateralis; DA, tractus dorso-arcopallialis; E, entopallium; HA, hyperpallium apicale; HD, hyperpallium densocellulare; GP, globus pallidus; M, mesopallium; MVL, mesopallium ventrolaterale; N, nidopallium; NCL, nidopallium caudolaterale; Rt, nucleus rotundus; StM, striatum mediale; TeO, tectum opticum.

For NCL, the intended track positions were AP +5.5 and ML ±7.5. Unfortunately, we were unable to recover the full track in NCL, but we were able to find the entry point of the electrodes at AP +5.5, ML +7.5 as intended. Based on our depth records, we believe the whole track was very likely to be within the targeted region (see Figure 7-3). For MVL and ENTO, the intended track positions were AP +9.5 and ML ± 6.0. Note that because MVL lies directly above ENTO, we used one set of electrodes to record from both areas by starting in MVL, and then advancing the electrodes forward until the start

of ENTO. The track position was at AP +9.5, ML -6.5, differing from the intended ML by 0.5mm.

7.3.2. Behavioural performance

The behavioural performance across all recording sessions for D4, depending on the implanted region, is shown in Figure 7-4.

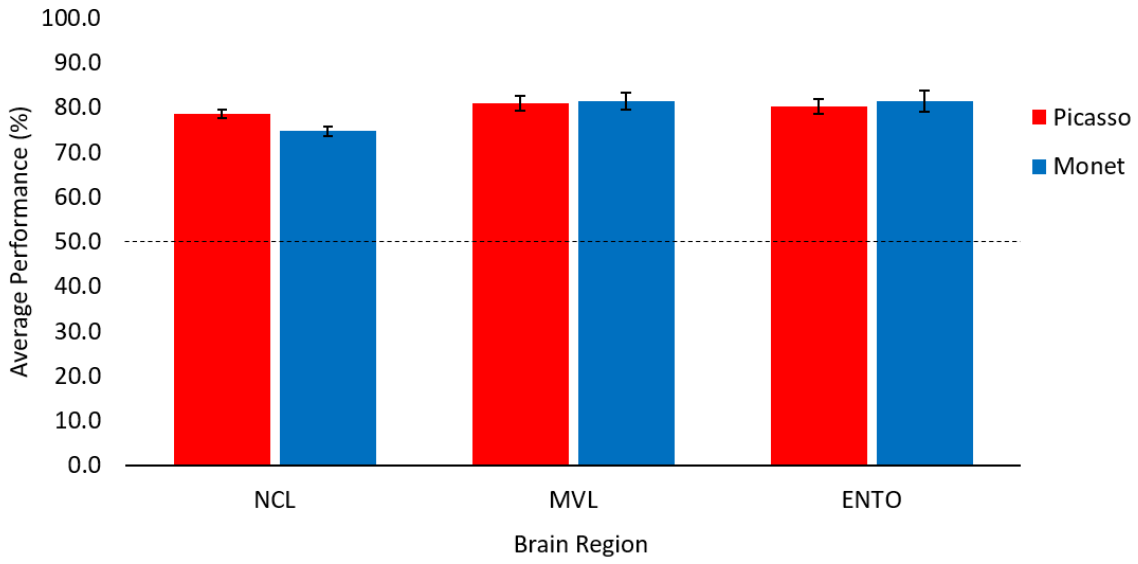

Figure 7-4: Behavioural performance.

The average performance (%) of D4 on both Picasso and Monet trials, across all three areas. The dashed line represents chance performance (50%). Error bars are ± 1 SEM.

D4 was able to correctly match Picasso and Monet paintings to their respective categories with fairly high accuracy. To see whether there were any differences in performance on Picasso and Monet trials, and between areas, we used a two-way ANOVA with artist (2: Picasso and Monet) and area (3: NCL, MVL, and ENTO) as factors

(Greenhouse-Geisser corrected). There was no significant difference in performance between Picasso and Monet trials, $F(1,115) = 0.25$, $p = 0.62$, nor a significant interaction effect between area and artist, $F(2,115) = 1.81$, $p = 0.17$. However, there was a significant difference in performance between the three areas, $F(2,115) = 6.67$, $p = 0.002$. Post hoc pairwise comparisons using the Bonferroni correction revealed that performance while recording from NCL ($M = 76.64$, $SE = 0.72$) was significantly lower compared to performance while recording from both MVL ($M = 81.21$, $SE = 1.36$, $p = 0.011$) and ENTO ($M = 80.76$, $SE = 1.33$, $p = 0.022$). The increase in accuracy from NCL to MVL and ENTO is likely due to the bird being exposed to more sessions by the time recordings were made in MVL and ENTO, as NCL was the first region we recorded from. Regardless, the increase in performance from NCL to MVL was small by approximately 4.6%, and D4 performed significantly above chance levels while recording from all three areas, all ts > 13.33, all ps < 0.001.

7.3.3. Neural filtering for visually responsive neurons

In total, we recorded from a total of 103 neurons, 63 from NCL, 20 from MVL, and 20 from ENTO. As with the previous studies outlined in Chapters 4-6, one way to compare neural activity during the categorisation task is to specifically analyse visually responsive neurons.

For a full breakdown of all visually responsive neurons, see Table 7-1. In NCL, 22/63 (34.9%) neurons were classified as visually responsive, while 9/20 (45.0%) in MVL and 20/20 (100.0%) in ENTO were classified as visually responsive. Of the 22 visually responsive NCL neurons, 13 neurons (59.1%) were excitatory to at least one of the stimuli, while the remaining 9 neurons (40.9%) were inhibitory. Of the excitatory NCL neurons, 11/13 (84.6%) selectively fired to Picasso stimuli, 1/13 (7.7%) selectively fired

to Monet stimuli, and 1/13 (7.7%) was non-selective in that it fired to both Picasso and Monet stimuli. Of the inhibitory NCL neurons, 5/9 (55.6%) selectively fired to Picasso stimuli, 3/9 (33.3%) selectively fired to Monet stimuli, and 1/9 (11.1%) was non-selective in that is fired to both Picasso and Monet stimuli. Due to the low numbers of excitatory neurons that were selective to Monet stimuli, we were unable to analyse this subset of neurons.

Of the 9 MVL neurons, 6 (66.7%) were classified as excitatory and 2 neurons (22.2%) were inhibitory. Of the excitatory MVL neurons, 3/6 (50.0%) selectively fired to Picasso stimuli, 2/6 (33.3%) was non-selective in that it fired to both Picasso and Monet stimuli, and 1/6 (16.7%) was selective to Monet stimuli. Of the inhibitory MVL neurons, 1/2 (50.0%) selectively fired to Picasso stimuli and 1/2 (50.0%) selectively fired to Monet stimuli. The final MVL neuron (11.1%) was inhibitory to Picasso paintings but excitatory to Monet paintings, and thus was included in both excitatory and inhibitory neuron counts. However, due to the low numbers of neurons recorded from MVL, we restricted all subsequent MVL analyses to excitatory neurons that were selective for Picasso stimuli.

Of the 20 ENTO neurons, all 20 (100.0%) neurons were inhibitory to at least one of the stimuli. Of these inhibitory neurons, 2/20 (10.0%) selectively fired to Monet stimuli, and 18/20 (90.0%) were non-selective in that they fired to both Picasso and Monet stimuli. Due to the low numbers of selective neurons in ENTO, we were unable to analyse this subset of neurons any further.

Table 7-1

Breakdown of Visually Responsive Neurons Across All Areas

	Excitatory Neurons				Inhibitory Neurons				Both		
	en	ne	ee	Total	in	ni	ii	Total	ei	ie	Total
NCL	11 (84.6%)	1 (7.7%)	1 (7.7%)	13	5 (55.6%)	3 (33.3%)	1 (11.1%)	9	0	0	0
MVL	3 (50.0%)	1 (16.7%)	2 (33.3%)	6	1 (50.0%)	1 (50.0%)	0	2	0	1 (100%)	1
ENTO	0	0	0	0	0	2 (10.0%)	18 (90.0%)	20	0	0	0

Note: e = excitatory; i = inhibitory; n = not significant from baseline. The first letter in each pair refers to the type of activity on Picasso trials, and the second letter refers to the type of activity on Monet trials.

7.3.4. Population profiles for visually responsive neurons

We constructed population profiles of visually responsive neurons in NCL and MVL, depending on whether they were selective to Picasso or Monet stimuli in the stimulus period, by averaging across all Picasso trials and across all Monet trials for each neuron. To see if there was any difference in neural activity to the Picasso and Monet categories, we used a two-way repeated-measures ANOVA with artist (Picasso vs Monet) and bin (6 bins for each of the periods; the middle 300 ms of the ITI, the 300 ms ready period, the middle 300 ms of the pause period, the 300 ms stimulus period, the middle 300 ms of the delay period, the 300 ms comparison period, and the middle 300 ms of the reward period, respectively) as factors, with repeated measures over stimuli and bins (Greenhouse-Geisser corrected). Note that we do not report results for any main effects of bin, as any significant value just reflects variations in the firing rates from one bin to the next, and is generally not of interest, but we do report interaction effects between artist and bin.

The population profile for Picasso-selective NCL excitatory neurons (NCL PE) is shown in Figure 7-5a. There was a significant main effect of artist in the stimulus period, $F(1,10) = 43.43$, $p < 0.001$, with greater excitatory activity on Picasso trials compared to Monet trials, but not in the other six periods, all Fs < 4.23, all ps > 0.067. There was also a significant interaction effect between artist and bin in the stimulus period, $F(5,50) = 3.14$, $p = 0.049$, with activity on Picasso trials being greater than activity on Monet trials at the start of the stimulus period, but rapidly decreasing in activity towards the end of the period, but not in the other six periods, all Fs < 2.48, all ps > 0.074.

The population profile for Picasso-selective NCL inhibitory neurons (NCL PI) is shown in Figure 7-5b. There was no significant main effect of artist in any of the seven

periods, all Fs < 5.07, all ps > 0.087, nor a significant interaction effect between artist and bin in any of the seven periods, all Fs < 2.61, all ps > 0.15.

The population profiles for Monet-selective NCL inhibitory neurons (NCL MI) is shown in Figure 7-5c. There was a significant main effect of artist in the ITI, $F(1,2) = 50.40$, $p = 0.019$, with more activity on Monet trials than Picasso trials in the second half of the ITI. While we should not see any difference in activity during the ITI between Monet and Picasso trials, as birds do not know which trial type it will be before the stimulus period, it is likely that this difference is due to the low number of inhibitory neurons that were Monet-selective (3) included in the analysis. There was also a significant main effect of artist in the stimulus period, $F(1,2) = 353.06$, $p = 0.003$, with greater activity to Picasso stimuli compared to Monet stimuli, which was more inhibitory. A significant main effect of artist was also found in the reward period, $F(1,2) = 22.03$, $p = 0.043$, with greater activity to Monet stimuli than to Picasso stimuli, but not in the other four periods, all Fs < 3.55, all ps > 0.2. There was also no significant interaction effect between artist and bin in any of the seven periods, all Fs < 1.85, all ps > 0.29.

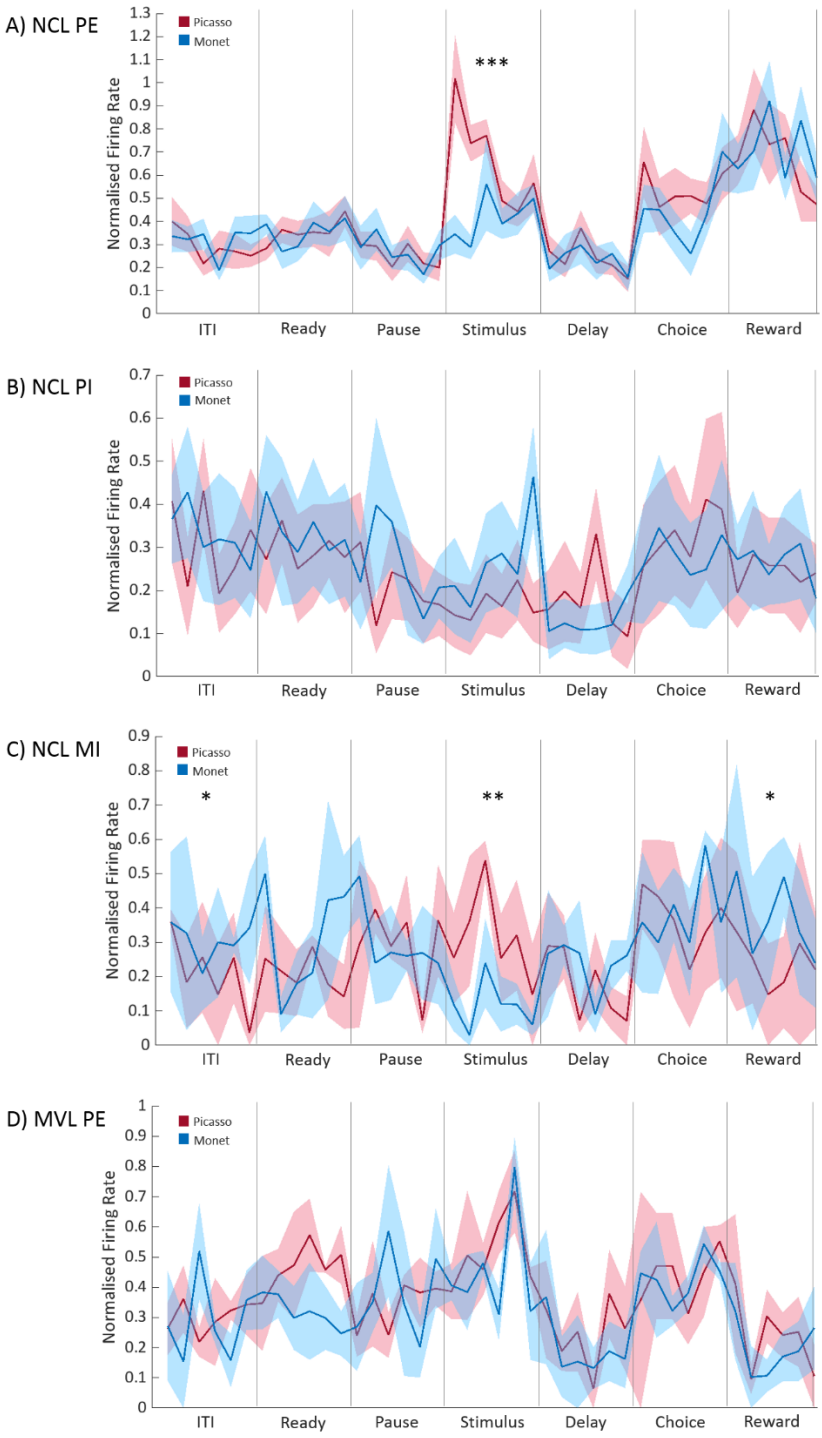

Figure 7-5: The population profiles for visually responsive neurons.

The population profiles for visually responsive NCL neurons (A-C) and MVL neurons (D). PE, Picasso-selective excitatory neurons; PI, Picasso-selective inhibitory neurons; MI, Monet-selective inhibitory neurons. Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval. * $p < .05$, ** $p < .01$, *** $p < .001$.

The population profile for Picasso-selective MVL excitatory neurons (MVL PE) is shown in Figure 7-5d. There was no significant main effect of artist in any of the seven periods, all Fs < 14.67, all ps > 0.062, nor any significant interaction effects between artist and bin in any of the seven periods, all Fs < 1.87, all ps > 0.28.

7.3.5. Category effects in the stimulus period

In NCL, both Picasso-selective excitatory (PE) neurons and Monet-selective inhibitory (MI) neurons displayed a significant main effect of artist in the stimulus period, in that activity to Picasso paintings was significantly different than activity to Monet paintings. To further examine the differences in activity between Picasso and Monet stimuli, we decided to separate trials even further into each of the four Picasso and Monet stimuli. While only two of the four conditions (PE and MI in NCL) displayed a significant main effect of artist, we also decided to examine the two non-significant conditions (PI in NCL and PE in MVL) in the same way. We then used a three-way repeated-measures ANOVA with artist (2: Picasso vs Monet), painting (4: four paintings within each category), and bin (6 bins for the 300 ms stimulus period) as factors, with repeated measures over stimuli and bins (Greenhouse-Geisser corrected). Note that we do not report results for any main effects of bin, nor any interaction effects with bin, as any significant value just reflects variations in the firing rates from one bin to the next and is generally not of interest.

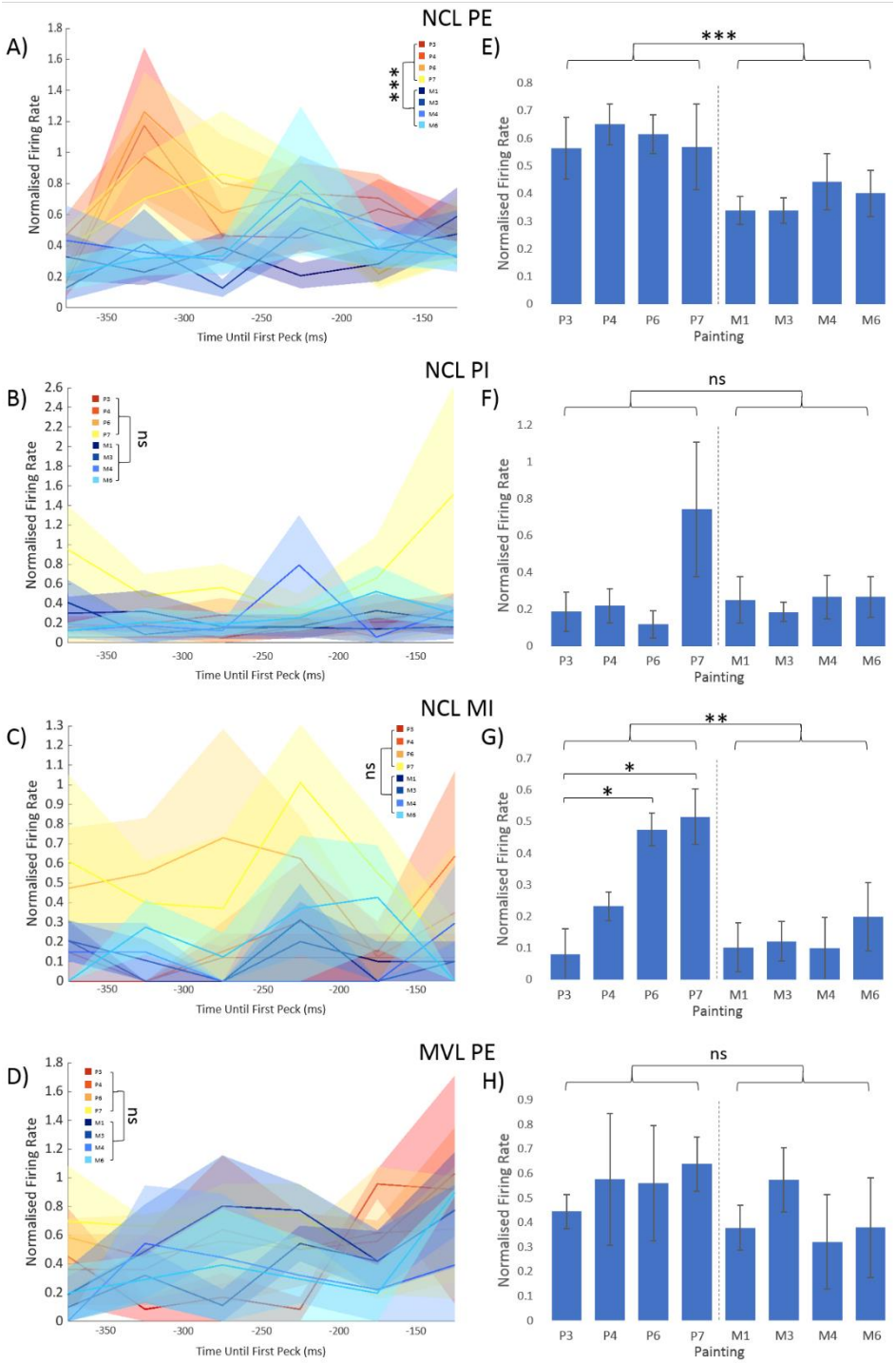

Figure 7-6: Category effects based on individual paintings in the stimulus period.

The population profiles based on individual paintings in the stimulus period (A-D) and averaged activity within the stimulus period to each individual painting (E-G), for visually selective neurons. PE, Picasso-selective excitatory neurons; PI, Picasso-selective inhibitory neurons; MI, Monet-selective inhibitory neurons. For A-D, shaded bands represent ±1 SEM. For E-G, dashed line represents the category boundary, and error bars are ±1 SEM. ITI, intertrial interval. * $p < .05$, ** $p < .01$, *** $p < .001$.

The population profile based on individual paintings for NCL PE neurons is shown in Figure 7-6a. In the stimulus period, there was a significant main effect of artist, $F(1,10) = 34.27$, $p < 0.001$, but there was no significant main effect of painting, $F(3,30) = 0.25$, $p = 0.75$, which suggests that within each category (Picasso and Monet), all paintings were perceived similarly, but between categories, they were perceived as different, i.e. a category effect. There was also no significant interaction effect between artist and painting, $F(3,30) = 0.34$, $p = 0.77$. When we average the activity across the 6 bins of the stimulus period for each painting (Figure 7-6e), a significant difference between the two categories is also found, $F(1,86) = 11.78$, $p = 0.001$, as well as a lack of significant difference between individual paintings within each category, both Fs < 0.48, both ps > 0.70.

The population profile based on individual paintings for NCL PI neurons is shown in Figure 7-6b. In the stimulus period, there was no significant main effect of painting, $F(1,4) = 0.43$, $p = 0.55$, nor a significant main effect of painting, $F(3,12) = 2.03$, $p = 0.21$, nor a significant interaction effect between artist and painting, $F(3,12) = 1.70$, $p = 0.26$, indicating a lack of a category effect in NCL PI neurons. A lack of category effect is also reflected in the average activity across the 6 bins of the stimulus period (Figure 7-6f), with no significant difference between categories, $F(1,38) = 0.40$, $p = 0.53$, nor within each category, both Fs < 2.07, both ps > 0.15.

The population profile based on individual paintings for NCL MI neurons is shown in Figure 7-6c. In the stimulus period, there was no significant main effect of artist, $F(1,2) = 7.44$, $p = 0.11$, nor a significant main effect of painting, $F(3,6) = 4.21$, $p = 0.15$, nor a significant interaction effect between artist and painting, $F(5,10) = 0.81$, $p = 0.48$, indicating a lack of a category effect in NCL MI neurons. However, when we averaged

activity across the 6 bins of the stimulus period (Figure 7-6g), we found a significant difference between categories, $F(1,22) = 7.19$, $p = 0.014$. We also found that there was a significant difference between activity to individual paintings within the Picasso category, $F(3,8) = 8.92$, $p = 0.006$, with post hoc pairwise comparisons using the Bonferroni correction revealing a significant difference between painting P1 ($M = 0.081$, $SE = 0.081$) and P6 ($M = 0.47$, $SE = 0.052$, $p = 0.022$), and painting P1 and P7 ($M = 0.52$, $SE = 0.088$, $p = 0.013$). There were no differences in activity to individual paintings within the Monet category, $F(3,8) = 0.28$, $p = 0.84$.

The population profile based on individual paintings for MVL PE neurons is shown in Figure 7-6d. In the stimulus period, there was no significant main effect of artist, $F(1,2) = 2.47$, $p = 0.26$, nor a significant main effect of painting, $F(3,6) = 0.20$, $p = 0.76$, nor a significant interaction effect between artist and painting, $F(5,10) = 8.74$, $p = 0.081$, indicating a lack of a category effect in MVL PE neurons. A lack of category effect is also reflected in the average activity across the 6 bins of the stimulus period (Figure 7-6h), with no significant difference between categories, $F(1,22) = 1.59$, $p = 0.22$, nor within each category, both Fs < 0.47, both ps > 0.71.

7.3.6. Filtering for delay-sensitive neurons

While our previous studies (Chapters 4-6) have solely compared neural activity during categorisation for visually responsive neurons, the paradigm used in the current study also enables us to compare the neural activity of delay neurons. We wanted to investigate whether categorical information is processed in delay-sensitive neurons, that is, neurons which display significantly different activity in the delay period compared to baseline ITI. Note that a neuron could be visually responsive but not delay-sensitive, and vice versa.

As with visually responsive neurons, delay-sensitive neurons were labelled by using a two-way repeated-measures ANOVA with period (the middle 5 s of the ITI and the 3 s delay period) and stimuli (words and nonwords) as factors, with repeated measures over stimuli (Greenhouse-Geisser corrected). If a neuron showed a significant difference in firing rate between the ITI and delay period for at least one of the stimuli (i.e. a main effect of period), then the neuron was labelled as being delay-sensitive.

Table 7-2

Breakdown of Delay-Sensitive Neurons Across All Areas

	Excitatory Neurons				Inhibitory Neurons				Both		
	en	ne	ee	Total	in	ni	ii	Total	ei	ie	Total
NCL	2 (50.0%)	1 (25.0%)	1 (25.0%)	4	7 (24.1%)	7 (24.1%)	15 (51.7%)	29	0	0	0
MVL	1 (100%)	0	0	1	1 (14.3%)	1 (14.3%)	5 (71.4%)	7	0	0	0
ENTO	0	0	0	0	0	0	20 (100%)	20	0	0	0

Note: e = excitatory; i = inhibitory; n = not significant from baseline. The first letter in each pair refers to the type of activity on Picasso trials, and the second letter refers to the type of activity on Monet trials.

For a full breakdown of all delay-sensitive neurons, see Table 7-2. In NCL, 33/63 (52.4%) neurons were classified as delay-sensitive, while 8/20 (40.0%) in MVL and 20/20 (100.0%) in ENTO were classified as delay-sensitive. Of the 33 NCL neurons, 29 (87.9%) were inhibitory to at least one of the stimuli, while the remaining 4 neurons (12.1%) were excitatory. Of the 29 inhibitory neurons, 7/29 (24.1%) selectively fired to Picasso stimuli, 7/29 (24.1%) selectively fired to Monet stimuli, and 15/29 (51.7%) were non-selective in that they fired to both Picasso and Monet stimuli. Of the 4 excitatory neurons, 2/4 (50.0%) selectively fired to Picasso stimuli, 1/4 (25.0%) selectively fired to Monet stimuli, and 1/4 (25.0%) were non-selective. Due to the low numbers of Picasso- and Monet-selective excitatory neurons, subsequent analyses were restricted to inhibitory NCL neurons.

Of the 8 MVL neurons, 7 (87.5%) were inhibitory to at least one of the stimuli, while one neuron (12.5%) was excitatory. Of the 7 inhibitory neurons, 1/7 (14.3%) selectively fired to Picasso stimuli, 1/7 (14.3%) selectively fired to Monet stimuli, and 5/7 (71.4%) were non-selective in that they fired to both Picasso and Monet stimuli. The single excitatory neuron selectively fired to Picasso stimuli. Due to the low numbers of Picasso- and Monet-selective excitatory and inhibitory neurons, no delay period analyses could be performed for MVL neurons.

Of the 20 ENTO neurons, all 20 (100.0%) were inhibitory to at least one of the stimuli. Of the 20 inhibitory neurons, all were non-selective in that they fired to both Picasso and Monet stimuli. Due to a lack of Picasso- and Monet- selective neurons, we were unable to perform any further analyses with the ENTO neurons.

7.3.7. Population profiles for delay-sensitive neurons

We constructed population profiles of delay-sensitive neurons in NCL, depending on whether they were selective to Picasso or Monet stimuli in the delay period, by averaging across all Picasso trials and across all Monet trials for each neuron. To see if there was any difference in neural activity to the Picasso and Monet categories, we used a two-way repeated-measures ANOVA with artist (Picasso vs Monet) and bin (6 bins for each of the periods; the middle 300 ms of the ITI, the 300 ms ready period, the middle 300 ms of the pause period, the 300 ms stimulus period, the middle 300 ms of the delay period, the 300 ms comparison period, and the middle 300 ms of the reward period, respectively) as factors, with repeated measures over stimuli and bins (Greenhouse-Geisser corrected). Note that we do not report results for any main effects of bin, as any significant value just reflects variations in the firing rates from one bin to the next, and is generally not of interest, but we do report interaction effects between artist and bin.

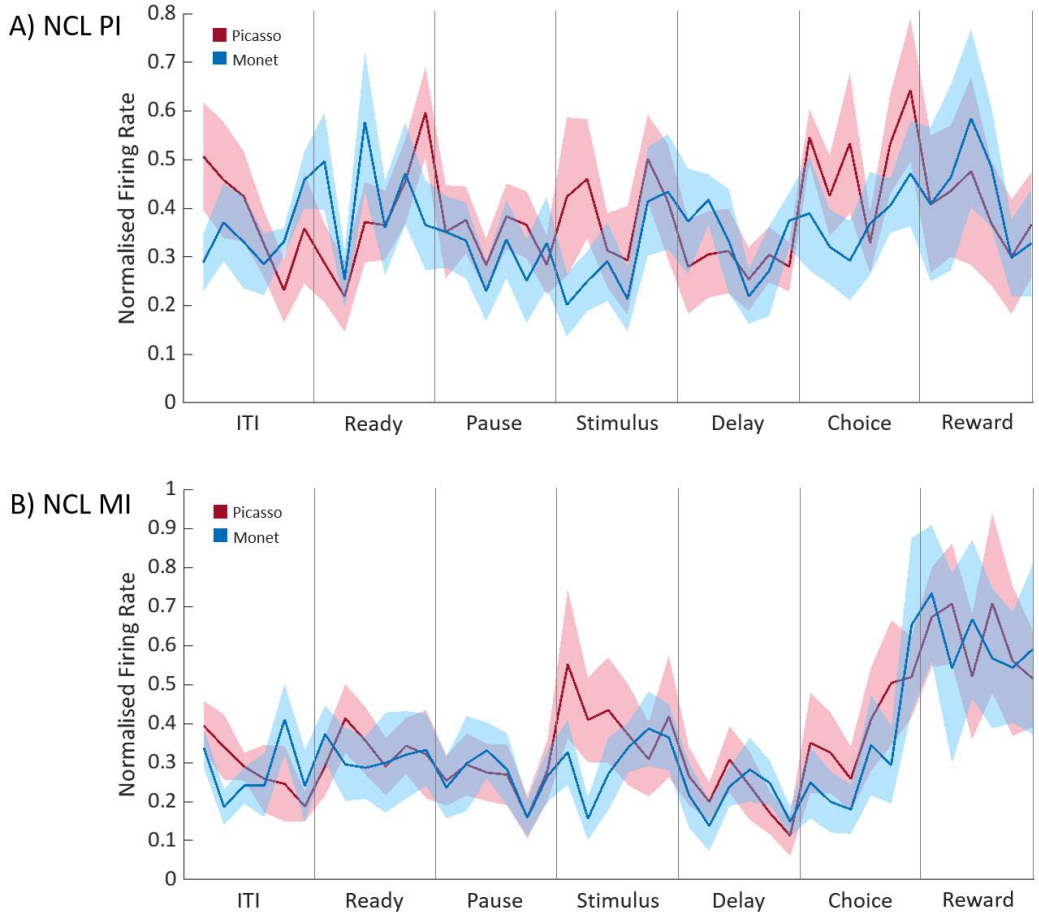

Figure 7-7: The population profiles for delay-sensitive neurons.

The population profiles for NCL delay-sensitive neurons. PI, Picasso-selective inhibitory neurons; MI, Monet-selective inhibitory neurons. Neuronal data was normalised against the maximum firing rate during the ITI, and bin width is 50 ms. Vertical lines separate the different periods of the task. Shaded bands represent ±1 SEM. ITI, intertrial interval. * $p < .05$, ** $p < .01$, *** $p < .001$.

The population profile for Picasso-selective inhibitory NCL neurons (NCL PI) is shown in Figure 7-7a. There was a significant main effect of artist in the comparison period, $F(1,6) = 6.50$, $p = 0.044$, with greater activity towards Picasso stimuli compared to Monet stimuli, but not in any of the other six periods, all Fs < 3.26, all ps > 0.12. There

was no significant interaction effect between artist and bin in any of the seven periods, all Fs < 1.82, all ps > 0.11.

The population profile for Monet-selective inhibitory NCL neurons (NCL MI) is shown in Figure 7-7b. There was no significant main effect of artist in any of the seven periods, all Fs < 4.72, all ps > 0.073. There was also no significant interaction effect between artist and bin in any of the seven periods, all Fs < 2.51, all ps > 0.088.

7.3.8. Category effects in the delay period

In NCL, neither of the Picasso-selective inhibitory (PI) neuron and Monet-selective inhibitory (MI) neuron populations displayed a significant main effect of artist in the delay period. Despite this lack of difference between activity to Picasso and Monet stimuli, as with visually responsive neurons, we decided to separate trials even further into each of the four Picasso and Monet stimuli. We used a three-way repeated-measures ANOVA with artist (2: Picasso vs Monet), painting (4: four paintings within each category), and bin (6 bins for the middle 300 ms of the delay period) as factors, with repeated measures over stimuli and bins (Greenhouse-Geisser corrected). Note that we do not report results for any main effects of bin, nor any interaction effects with bin, as any significant value just reflects variations in the firing rates from one bin to the next and is generally not of interest.

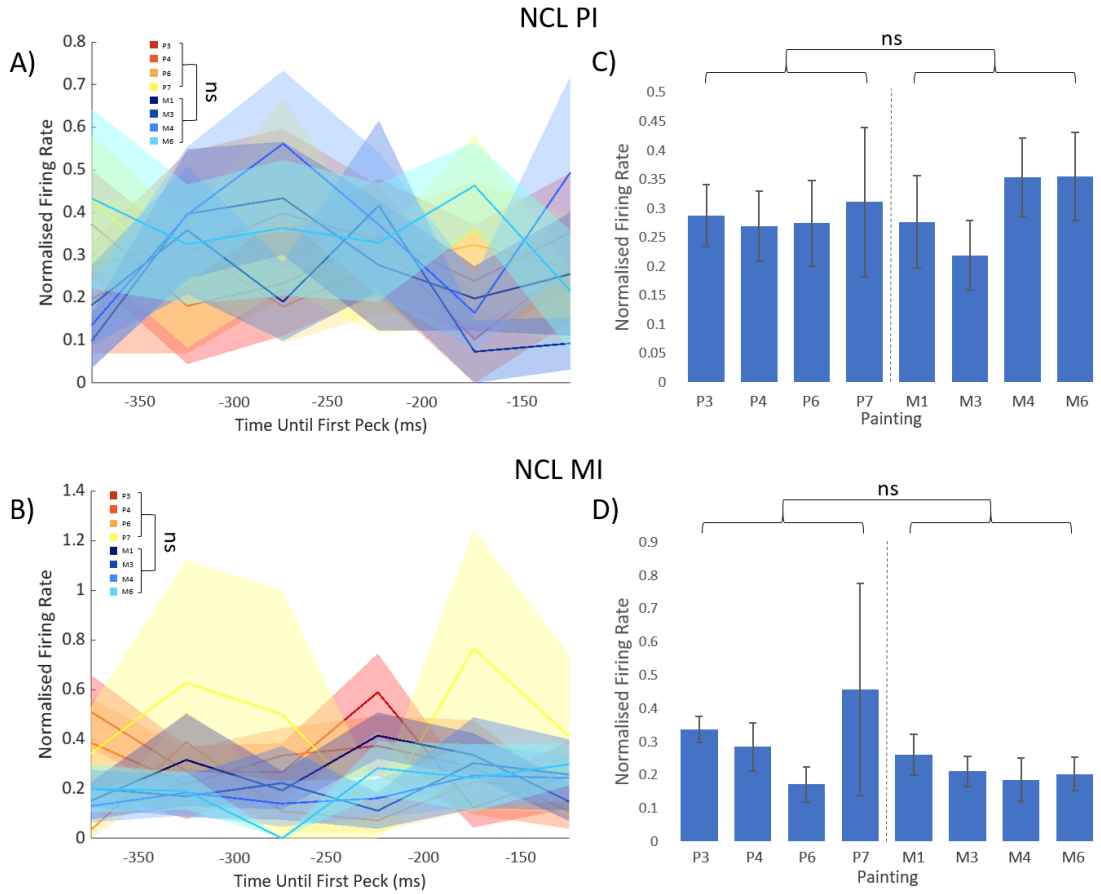

Figure 7-8: Category effects based on individual paintings in the delay period.

The population profiles based on individual paintings in the delay period (A and B) and averaged activity within the delay period to each individual painting (C and D), for delay-sensitive neurons. PI, Picasso-selective inhibitory neurons; MI, Monet-selective inhibitory neurons. For A and B, shaded bands represent ±1 SEM. For C and D, dashed line represents the category boundary, and error bars are ±1 SEM. ITI, intertrial interval. * p < .05, ** p < .01, *** p < .001.

The population profile based on individual paintings for NCL PI neurons is shown in Figure 7-8a. In the delay period, there was no significant main effect of artist, $F(1,6) = 0.13$, $p = 0.73$, nor a main effect of painting, $F(3,18) = 1.10$, $p = 0.37$, nor a significant interaction effect between artist and painting, $F(3,18) = 0.44$, $p = 0.66$, indicating a lack

of a category effect in NCL PI neurons. A lack of category effect is also reflected in the average activity across the 6 bins of the stimulus period (Figure 7-8b), with no significant difference between categories, $F(1,55) = 0.86$, $p = 0.77$, nor within each category, both Fs < 0.85, both ps > 0.48.

The population profile based on individual paintings for NCL MI neurons is shown in Figure 7-8c. In the delay period, there was no significant main effect of artist, $F(1,6) = 0.94$, $p = 0.37$, nor a main effect of painting, $F(3,18) = 0.55$, $p = 0.50$, nor a significant interaction effect between artist and painting, $F(3,18) = 0.40$, $p = 0.57$, indicating a lack of a category effect in NCL MI neurons. A lack of category effect is also reflected in the average activity across the 6 bins of the stimulus period (Figure 7-8d), with no significant difference between categories, $F(1,55) = 1.32$, $p = 0.26$, nor within each category, both Fs < 0.51, both ps > 0.68.

7.4. Discussion

7.4.1. Summary of findings – visually responsive neurons

In NCL, 22/63 (34.9%) neurons were classified as visually responsive, while 9/20 (45.0%) in MVL and 20/20 (100.0%) in ENTO were classified as visually responsive. Due to the low number of visually selective neurons in ENTO, neurons in this area were not used in the analysis. In both NCL and MVL, the majority of excitatory neurons selectively fired to Picasso stimuli, but not Monet stimuli (NCL PE: 84.6% and MVL PE: 50.0%, respectively). We were unable to analyse NCL and MVL excitatory neurons that fired selectively to Monet stimuli due to low numbers. While inhibitory MVL neurons were also excluded due to low numbers, the majority of inhibitory NCL neurons

selectively fired to Picasso stimuli (NCL PI: 55.6%), and a minority of inhibitory NCL neurons selectively fired to Monet stimuli (NCL MI: 33.3%). We further analysed these neurons using a two-way repeated-measures ANOVA (Greenhouse-Geisser corrected) to see if there was any difference in neural activity to the Picasso and Monet categories within each task period. In NCL PE neurons, we found that overall activity to Picasso stimuli was significantly greater than activity to Monet stimuli in the stimulus period, but there was no significant difference in activity to the two categories in NCL PI neurons. In NCL MI neurons, activity was significantly more inhibitory to Monet stimuli than Picasso stimuli in the stimulus period, but more excitatory to Monet stimuli in the reward period. Finally, activity to Monet and Picasso stimuli did not differ in MVL PE neurons.

We then separated trials into each of the four Picasso and Monet stimuli to examine the differences in activity both between and within the categories during the stimulus period, using a three-way repeated-measures ANOVA (Greenhouse-Geisser corrected). As with our original analyses, both NCL PI and MVL PE neurons did not show any between- or within-category differences in activity in the stimulus period. In NCL PE neurons, we found a significant difference in activity between the two categories, but no differences within the categories, indicating that the greater activity towards Picasso stimuli compared to Monet stimuli was consistent across all individual Picasso stimuli and Monet stimuli. On the other hand, in NCL MI neurons, while we did find a significant difference in activity between the two categories, we also found differences between individual stimuli within categories, in that NCL MI neurons showed greater inhibitory activity to stimulus P3 compared to both stimulus P6 and P7. However, there were no differences in activity to individual stimuli within the Monet category, indicating that NCL MI neurons responded in a similar way to all Monet paintings, and that the

significant difference in overall Monet and Picasso activity is likely driven by a difference between all Monet stimuli and P6 and P7 specifically.

7.4.2. Summary of findings – delay-sensitive neurons

In NCL, 33/63 (52.4%) neurons were classified as delay-responsive, while 8/20 (40.0%) in MVL and 20/20 (100.0%) in ENTO were classified as visually responsive. Due to the low numbers of delay-selective neurons in MVL and ENTO, neurons in these areas were not used in the analysis. In NCL, an equal number of inhibitory neurons were selective to Picasso stimuli and selective to Monet stimuli. We were unable to analyse delay-selective excitatory neurons in NCL due to low numbers. Using the same 2-way ANOVA described in section 7.4.1., we found that in both NCL PI and MI neurons, there was no significant difference in activity to Picasso stimuli compared to Monet stimuli. When we separated trials into each of the individual stimuli, we also found that there was a lack of within- and between-category differences in activity in the delay period for both NCL PI and MI neurons.

7.4.3. Preliminary conclusions on categorisation in NCL

We predicted that we would find more delay-sensitive neurons than visually responsive neurons in NCL. Indeed, more neurons were classed as being delay-sensitive (52.4%) than visually responsive (34.9%). Similarly, Freedman et al. (2003) found larger proportion of primate PFC neurons were category-sensitive during the delay period compared to the sample period. Freedman et al. (2003) argues that because PFC neurons are more sensitive to differences between categories in the delay period, the PFC more likely encodes information about category membership beyond basic visual properties. Thus, it is likely that NCL, the avian analogue of primate PFC, also encodes categorical information beyond the visual properties of the stimuli, i.e. category-reward associations.

Among visually responsive neurons, we found that NCL PE and MI neurons displayed differential activity towards Picasso and Monet stimuli within the stimulus period. In our previous study using Picasso and Monet paintings (Chapter 5) we did not find any differences in activity between the categories within the stimulus period in NCL, but rather in the reward period. We found differences in activity to Picasso and Monet stimuli in the reward period for NCL MI neurons, indicating that this subset of neurons may be using category-reward associations to differentiate between the two categories. However, as the analysis for NCL MI neurons is only based on three neurons, it is difficult to say whether the differences found in the reward period are an accurate reflection of NCL MI population activity, especially when we also found a significant difference in activity in the ITI, when our bird would not yet know what trial type was taking place. Furthermore, when we split trials into the four Picasso and four Monet stimuli, we found that NCL MI neurons did not respond in a similar way to all Picasso paintings. Activity to paintings P6 and P7 was significantly different to activity to painting P3, suggesting that differences in between-category activity is likely driven by a difference between all Monet stimuli and P6 and P7 specifically, rather than the Picasso category as a whole.

In NCL PE neurons, we found distinct between- and within-category effects in the stimulus period of visually responsive neurons. That is, these neurons discriminated between Picasso and Monet categories overall, but also responded to all Monet stimuli similarly, and all Picasso stimuli similarly, much like the category effects found in IT cortex neurons in Freedman et al. (2001; 2003). As Keller and Schoenfeld (1950) note, true categorisation requires an individual to both discriminate between two categories, but also to generalise within each category. Thus, in NCL PE neurons, it appears that neural activity during the stimulus period is reflective of true categorisation. The reason

that differences in activity appear within the stimulus period of the current study is likely because of a lack of differential reward outcomes. In Chapter 5, our S+/S- paradigm had differential reward outcomes (reward vs. no reward) associated with each category, while the current study has no differential rewards (correct responses to both categories result in the same reward. We know that NCL neurons essentially default to reward-based coding when there is the opportunity to do so, such as through differential rewards (Johnston et al., 2017a). When there is no opportunity for reward-based coding, like in the current study, NCL neurons can flexibly switch to more stimulus-based coding (Johnston et al., 2017b), in which they incorporate behavioural information with information about different stimulus-reward associations (Kirsch et al., 2009; Lengersdorf et al., 2014).

However, in NCL we would also expect differences in activity to the two categories to continue through the delay period and even into the reward. Category-sensitive PFC neurons in Freedman et al. (2003) displayed sustained activity through the delay period while IT cortex neurons did not, which the authors argue is evidence for the PFC being involved in categorisation beyond visual discrimination, and likely incorporates information about guiding behaviour during categorisation. It is possible that we did not find sustained activity through the delay period of visually responsive neurons because these neurons are not particularly involved in working memory; rather, NCL neurons which are visually responsive may actually be holding visual information from visual areas such as ENTO, which projects to NCL (Kröner & Güntürkün, 2009; Krützfeldt & Wild, 2005). Visual information received from visual areas might then be incorporated with categorical and behavioural information from delay sensitive NCL neurons to guide categorisation.

Since visually responsive neurons were not necessarily delay-sensitive, and vice versa, we also decided to classify neurons as being delay-sensitive based on whether activity during the delay period was different to baseline ITI activity. Among delay-sensitive neurons, we found that NCL PI and MI neurons did not display differential activity towards Picasso and Monet stimuli within the delay period. In Freedman et al. (2003), category effects were stronger in the delay period than the stimulus period in PFC neurons, and so we expected to find stronger category effects in the delay period of NCL neurons as well. However, the lack of category effects among our delay-sensitive neurons may be due to the fact that we only recorded from one bird, and only from the right hemisphere. In Chapter 5 we found a strong left hemisphere dominance for categorisation among NCL neurons. Therefore, the fact that we were only able to record from the right hemisphere may be why we did not find any category effects in delay-sensitive neurons, and only in two subsets of visually responsive neurons. Further investigation into the activity of left hemisphere neurons during the same DMC task is needed.

7.4.4. Preliminary conclusions on categorisation in MVL and ENTO

We predicted that we would find more visually responsive neurons than delay-sensitive neurons in ENTO and MVL. Indeed, more neurons were classed as being visually responsive (45.0%) than delay-sensitive (40.0%) in MVL. Similarly, Freedman et al. (2003) found a larger proportion of IT cortex neurons were category-sensitive in the stimulus period than in the delay period. Freedman et al. (2003) argues that because IT cortex neurons are more sensitive to category differences in the stimulus period, IT cortex more likely encodes differences in the visual properties of stimuli between categories. Thus, it is likely that MVL, the avian analogue of IT cortex, also encodes categorical information based on the visual properties of the stimuli within a category. However, in

ENTO, there was an equal amount of visually responsive and delay-sensitive neurons (100%). ENTO is thought to be an analogue of some portion of extrastriate cortex in primates, and thus is not an analogue of IT cortex per se. The fact that ENTO neurons appear to be equally visually responsive and delay-sensitive may be due to the fact that ENTO neurons incorporate information about visual properties and reward (Johnston et al., 2017a). Thus, ENTO neurons may encode categorical information based on visual properties like MVL, but also information about behavioural responses and reward like in NCL to some degree. Unfortunately, due to the fact that we could only recorded neurons from one bird, we did not find enough visually- or delay-selective neurons in ENTO to continue these analyses. We were also unable to analyse delay sensitive MVL neurons for the same reason.

Among visually responsive neurons, we found that MVL PE neurons did not display differential activity towards Picasso and Monet stimuli within the stimulus period. In Freedman et al. (2003), category effects were stronger in the stimulus period than in the delay period in IT cortex neurons, and so we expected to find stronger category effects in the stimulus period of MVL neurons as well. Like with delay sensitive NCL neurons, this is likely due to the fact that we were only able to record from one bird in the current study. While we only recorded from the right hemisphere of MVL, we did not expect to see the same left-hemispheric dominance as in NCL, as previously we did not find any hemispheric differences in MVL (see Chapter 5). Further investigation into the activity of MVL neurons from both hemispheres during the DMC task is needed.

CHAPTER 8 |

GENERAL DISCUSSION

8.1. Summary of Chapters

8.1.1. Delay activity in the visual Wulst

In this first study, we wanted to investigate the Wulst, the second main visual area in pigeons, as a potential area of interest (in addition to NCL and ENTO) for subsequent categorisation studies. We decided to examine the Wulst using single-unit electrophysiology while birds performed a simple delayed matching-to-sample (DMS) task, so that neural data from the Wulst could be compared to similar neural data from NCL and ENTO.

We trained two groups of birds on either the CO DMS or DO DMS task from Johnston et al. (2017a; 2017b), in which birds were either rewarded following correct matches to both stimuli (CO DMS) or only rewarded following correct matches to just one of the stimuli (DO DMS). Behaviourally, we found that birds performed equally well on both trial types when trained with CO conditions, but when trained on DO conditions, the birds performed better on rewarded trials than unrewarded trials. We argue that the difference in performance under DO conditions is due to the fact that one trial type is more motivating than the other; that is, rewarded stimuli likely motivate the birds to remember the stimulus during the delay more so than when the unrewarded stimulus is shown.

However, birds still do well on unrewarded trials as they must choose the correct comparison in order to advance the task.

On the neural level, neurons of birds who were trained on CO conditions showed significant differences in activity during the delay period compared to baseline ITI activity for both trial types. In previous studies, we found significant delay activity during both trial types in ENTO neurons in both CO and DO DMS trained birds (Colombo et al., 2001; Johnston et al., 2017a), which we have argued is evidence of sample coding (i.e. coding information about the to-be-remembered stimulus). Therefore, similar to neurons in the other intermediary visual area (ENTO), Wulst neurons are likely coding information about the to-be-remembered stimulus under CO conditions.

On the other hand, neurons of DO trained birds responded differently depending on whether they were excitatory or inhibitory. Inhibitory neurons displayed a similar pattern of activity to CO neurons, in that delay activity to both trial types was significantly different from baseline. However, unlike CO Wulst neurons, inhibitory DO neurons also displayed significantly different activity between rewarded and unrewarded trials during the delay period, indicating that these neurons were also using reward information to distinguish between the trial types. Previously, we also found reward-modulated activity in ENTO neurons under DO conditions (Johnston et al., 2017a). Most strikingly, excitatory DO Wulst neurons seem to be even further influenced by reward information, as in these neurons, only delay activity to rewarded trials was different from baseline, while unrewarded trials did not differ between baseline and delay activity. Delay activity to just the rewarded stimuli is a pattern typical of NCL, the avian analogue of primate PFC, an area heavily involved with reward-related processing (Johnston et al., 2017a).

The fact that we found reward modulation in inhibitory DO neurons and explicit reward coding in excitatory DO neurons indicates that the Wulst is much more involved in reward processing than other visual areas like ENTO. We argue that Wulst neurons display a high degree of flexibility in their encoding in that they can encode both sample and reward information, but may default to one type of coding over the other based on the demands of the task (i.e. the reward conditions). While there is evidence that both NCL and ENTO also incorporate other types of information during the delay, these two areas seem to have more distinct primary processing functions (visual-based information in ENTO, and reward-based information in NCL) than the Wulst. Due to the flexibility of the Wulst, it is more difficult for us to predict and understand what information Wulst neurons will be processing during categorisation, and thus we decided that we would not continue to record from Wulst neurons during subsequent categorisation studies.

8.1.2. Categorisation of English words and nonwords

In the first of four categorisation studies we trained birds on a word versus nonword discrimination task. The semi-symbolic discrimination task required birds to peck the four-letter string presented if it was a real English word that they had learned or peck an asterisk symbol located either above or below the four-letter string if it was a nonword. Behaviourally, we found that all birds were able to discriminate words from nonwords at a high level. Furthermore, we found some correlation between behavioural performance and the bigram frequency of words. Two birds showed a significant correlation between bigram frequency and accuracy (the NCL bird with the small vocabulary and the ENTO bird with the large vocabulary), one bird approached a significant correlation (the ENTO bird with the small vocabulary), and the final bird showed no correlation (the NCL bird with the large vocabulary).

On the neural level, we found no differences in overall activity to words and nonwords in NCL and ENTO neurons, in any period of the task. We expected to at least find differences between the two categories in a visual area (ENTO) as the two categories should differ visually (i.e. nonwords always only contained one vowel). Unfortunately, it is possible that the paradigm used has prevented us from observing any differences in activity caused by visual differences in words and nonwords. On word trials, the birds were able to assess the string and then move towards it; whereas on nonwords trials, the birds had to assess the string and then move towards the asterisk. As we are only able to measure activity just before the birds pecked, we may have missed the window in which the birds were assessing the string and where we would expect to see differences encoded in a visual area.

In terms of NCL activity, we expected more behaviourally-driven categorisation rather than visual-based categorisation. Interestingly, NCL neural activity seemed to be tied more to bigram frequency than ENTO activity, contrary to expectations. When we added bigram frequency as a factor, we did find a difference in activity between nonwords and high bigram frequency words in the reward period of the NCL bird with the smaller vocabulary. That is, NCL neurons in this bird appeared to be differentially encoding rewards associated with different categories, much like NCL neurons in Kirsch et al. (2009). However, differential reward coding only occurred between nonwords and high bigram frequency words, the two categories that were most distinct from one another. Activity to words with a low or medium bigram frequency was no different from either nonwords or high bigram frequency words, suggesting that at least for the NCL bird with the small vocab, bigram frequency was useful in distinguishing words both behaviourally and neurally.

We initially expected bigram frequency to have more visual associations in that it would be an indicator of visual differences between words and nonwords. However, it seems that rather than signalling visual differences in the two categories, bigram frequency indicates which behavioural decision must be executed. Indeed behaviourally, bigram frequency is correlated with accuracy in most birds, with the exception of the NCL bird with the larger vocabulary (Q43). Q43 does not seem to use bigram frequency to aid behavioural decisions and may be why we did not find similar differences in activity to different bigram frequencies in their neural activity as we did with the other NCL bird. It may also be that birds who have larger vocabularies have better memory in general, and rather than using bigram frequency to distinguish words from nonwords, those birds are able to memorise and recognise which are 'words' from their vocabularies. Furthermore, bigram frequency may be useful during learning (as seen in the behavioural data of Scarf et al., 2016), but once learning ceases, birds then switch to a more memory-based discrimination.

8.1.3. Categorisation of Monet and Picasso paintings

In the second of four categorisation studies, we trained birds to discriminate a set of Monet paintings from a set of Picasso paintings. An S+/S- paradigm was implemented, in which half the birds were trained to peck Picasso paintings and refrain from responding to Monet paintings (P+), and vice versa for the other half of the birds (M+). In addition to NCL and ENTO, we recorded from MVL, a higher-order visual area more recently considered to be analogous to primate IT cortex (Stacho et al., 2016).

Behaviourally, we found that all birds highly discriminated between Monet and Picasso paintings, regardless of which set of paintings they had been trained to peck (i.e. M+ or P+). On the neural level, we found no differences in activity between S+ and S-

trials in the overall NCL population but did find a difference in the stimulus period for the overall population of ENTO and MVL neurons. In NCL, we expected to find differential activity in the reward period, especially considering our S+/S- paradigm is based on differential rewards. While a difference in activity to S+ and S- trials emerges as the reward period progresses, the difference in activity did not reach significance. The fact that ENTO and MVL neurons displayed significant differences in activity to Monet and Picasso paintings during the stimulus period indicates these visual areas encode differences in the visual properties of each category.

As a further analysis, we examined whether there were any hemispheric differences in firing patterns within each region. While we had not found any overall differences in activity in NCL neurons, we now found a significant left-hemisphere bias, in which left hemisphere NCL neurons displayed significantly different activity between categories in the reward period, but right hemisphere NCL neurons did not. We also found the same left-hemisphere bias in ENTO neurons in both the stimulus and reward period. In birds, a left hemisphere dominance is well-documented for both visual categorisation (Vallortigara, 2000; Vallortigara & Andrew, 1994; Yamazaki et al., 2007) and reward-related discrimination (Güntürkün et al., 2000; Güntürkün & Kesch, 1987), particularly when discriminating ecologically important stimuli such as food (Rogers, 2008). Thus, the differences in activity to S+ and S- trials during the reward period in left hemisphere NCL neurons indicates that the left NCL is specialised for encoding reward-category differences. Furthermore, the differences in activity to S+ and S- trials during the stimulus period in left hemisphere ENTO suggests that left ENTO is specialised for encoding visual category differences, particularly local features (whereas the right hemisphere processes global features; see Yamazaki et al., 2007). However, as we also found

differences in activity during the reward period in left ENTO neurons, it is likely that ENTO also incorporates reward-related information to help guide categorisation as has been suggested by previous literature (see Johnston et al., 2017a; Verhaal et al., 2012).

While we found strong hemispheric differences in both NCL and ENTO neural activity, we did not find any hemispheric differences in MVL. Both hemispheres displayed the same differential activity during the stimulus period that was found in the overall MVL population. We argue that the lack of hemispheric differences in MVL reflects purely visual categorisation, especially as there appears to be no reward modulation present in MVL neurons. While categorisation based on local features of stimuli may be more present in ENTO neurons, information about both local and global features are likely processed in tandem by MVL.

8.1.4. Relevant and irrelevant features during categorisation

In the third of four categorisation studies we trained birds to discriminate between two categories of complex exemplars. Each exemplar from each category was made up of four smaller stimuli, two of which were always unique to the category (relevant features), and two which were shared between the categories (irrelevant features). We used a simultaneous matching-to-category (SMC) paradigm which presented birds with an exemplar belonging to one of two categories. Birds were required to peck a patterned choice stimulus that was mapped to the category shown and were awarded the same reward for correct responses to both categories.

Behaviourally, our birds were able to achieve a high level of performance in discriminating between the two categories. However, there were some interesting differences in performance, especially when compared to the performance of birds trained on a similar paradigm in Castro and Wasserman (2014). Our birds preferred pecking B-

relevant stimuli from the start of training compared to B-irrelevant stimuli, despite never having been exposed to either set of stimuli. On the other hand, birds did not have a preference for pecking A-relevant stimuli over A-irrelevant stimuli at the start of training. As training progressed, performance to A and B exemplars equally increased to criteria. By the end of training, birds did tend to peck A-relevant stimuli significantly more than A-irrelevant stimuli, but the divergence in pecking never reached the same level as between B-relevant and irrelevant stimuli. We argue that the differences in pecking behaviour towards A and B exemplars is likely caused by visual differences between the relevant and irrelevant stimuli within each set. All A stimuli are blue/purple, whereas in B exemplars, the blue/purple irrelevant stimuli are contrasted against the more yellow/orange B-relevant stimuli. It may be that because all A stimuli are so similar in hue, birds find it hard to differentiate between the stimuli at the start of training, but because B-relevant stimuli are so distinct from the B-irrelevant stimuli, birds prefer to peck the more novel colour at the start of training.

We analysed our neural population data on three different levels: category, relevance, and stimulus. At the category level, we did not find any differences in neural activity to the two categories in ENTO neurons. In NCL, we found a significant difference in activity to the two categories in the stimulus period for excitatory neurons, and in the choice period for inhibitory neurons. While we did not find any differences in the reward period as with our previous experiments, we argue that the lack of reward differences is likely caused by the contingency of the SMC paradigm. In the SMC task, correct responses to either category results in the same reward, and due to the flexible nature of the NCL (see Johnston et al., 2017a; 2017b), the NCL may switch away from reward processing to more visually based processing. In fact, we argue that for excitatory NCL neurons, it is

likely that these neurons are encoding colour differences between relevant and irrelevant stimuli within each category. On the other hand, we posit that inhibitory NCL neurons may be encoding differential responses associated with each category, which is why differences in activity emerge in the choice period for these neurons.

At the relevance level, we did not find any differences in activity within NCL neurons. Due to low numbers of analysable neurons in ENTO at this level, we decided to analyse our neurons at the single neuron level using Bayesian mixed effects modelling. At the single neuron level, we now found differences in firing rates to B-relevant stimuli compared to A-relevant stimuli in the choice period for both ENTO and NCL neurons. Like our behavioural data, we posit that the differences in firing rates between these conditions is caused by the distinctiveness of the B-relevant stimuli compared to A-relevant and irrelevant stimuli. Thus, at the relevancy level, NCL is likely associating the colour differences with behavioural relevance; that is, the blue/purple A-relevant stimuli are associated with a particular choice pattern key (dependent on which pattern had been mapped to each category during training), and the yellow/orange B-relevant stimuli are associated with the other choice pattern key. In ENTO, we would have expected differences to emerge in the stimulus period when the exemplars are first presented rather than the choice period. However, we argue that the birds do not look at the whole exemplar until the choice period commences, at which point ENTO neurons are able to encode visual differences in the stimuli.

Finally, at the stimulus level, we found no differences in activity between the two categories for NCL neurons. We did not expect to find differences at the stimulus level for NCL as NCL is not likely to encode visual differences beyond the relevancy level (i.e. purely visual feature differences). Unfortunately, we had too few ENTO neurons

available to analyse at the stimulus level and could not analyse ENTO neurons at the single neuron level due to time restrictions.

8.1.5. Delayed category matching of Monet and Picasso paintings

In the final of four categorisation studies, we trained birds to discriminate between a subset of the same Monet and Picasso paintings used in Chapter 5, as our birds had previously found these stimuli easy to discriminate. Unlike our previous experiment, we used a delayed matching-to-category (DMC) paradigm, in which birds were presented with one of the painting stimuli followed by a delay. The birds were then presented with one Monet and one Picasso painting and had to peck the painting that was from the same category as the one they had seen previously.

Behaviourally, our bird performed highly on the discrimination, although accuracy was slightly lower when recording from NCL than in ENTO and MVL. However, we argue that the increase in performance when recording from MVL and ENTO is simply because we recorded from NCL first, and then reimplanted the bird to record from MVL and ENTO, and thus is just an artifact of the number of sessions the bird had experienced by that point. For our neural data, we analysed neurons in two different ways; whether they were visually responsive (the same way we had in our other studies) or whether they were delay-sensitive, i.e. whether they displayed significantly different activity during the delay period compared to baseline ITI activity.

We found more delay-sensitive neurons in NCL, more visually responsive neurons in MVL, and an equal number of visually responsive and delay-sensitive neurons in ENTO. The fact that we find more delay-sensitive neurons in NCL corresponds with the idea that NCL is involved in guiding behaviour, and in terms of categorisation, forms associations between category membership and behavioural outcomes. On the other hand, the fact that

we find more visually responsive neurons in MVL corresponds with the idea that MVL is involved in visual processing, and in terms of categorisation, encodes differences in the visual properties of categories to aid discrimination. Similarly, ENTO appears to also be involved with encoding visual differences, but also encodes some behaviourally relevant differences (e.g., reward).

In terms of category effects, we found that visually responsive NCL neurons displayed significant category effects, but not delay-sensitive NCL neurons. Monet-selective inhibitory (MI) neurons displayed significant differences in activity to Monet and Picasso paintings in both the stimulus and reward periods, while Picasso-selective excitatory (PE) neurons displayed significant differential activity in just the stimulus period. The fact that MI and PE neurons differentiate between the sets of paintings in the stimulus period indicates once again that NCL encodes visual differences between the paintings. Furthermore, the fact that MI neurons also differentiate between the categories in the reward period shows that the visual differences NCL encodes are likely being associated with different rewards, despite there being no differences in reward at face value. Although PE neurons do not differentiate between categories in the reward period, a slight difference in activity can be seen in the choice period, which may also reflect associations being made between visual information and behavioural outcomes. Unfortunately, it is difficult to draw firm conclusions from our data as we only recorded from a single bird.

Interestingly, we found no category effects in delay sensitive NCL neurons, nor in either subset of MVL and ENTO neurons. While we would expect strong category effects in these areas, it is likely that the small sample size has prevented us from seeing any differences, as well as the fact that we only recorded from the right hemisphere of each of these areas.

8.2. Mechanisms of Categorisation in NCL, ENTO, and MVL

The aim of this thesis was to understand how neurons in NCL and ENTO are involved in the categorisation process, and how neurons from these areas use reward/behaviour-driven information and visual information about a category during discrimination. Based on our findings (Chapters 4-7), we will propose a potential model of categorisation within each area and theorise how the areas interact and share information. We will also include MVL in our theorised model, although since we do not have extensive data from this area, our discussion of this area will be more speculative.

8.2.1. The role of NCL in categorisation

Based on previous literature discussed in Chapter 1, we expected NCL to be heavily involved in using reward and relevant behavioural information to distinguish between categories and then execute an appropriate behavioural decision. In our first categorisation study, we found evidence of NCL neurons encoding rewards associated with different categories, despite there being no differences in the rewards themselves. Particularly, in the case of our word/nonword discrimination task, the bird that had not yet memorised their vocabulary relied on using bigram frequency to aid categorisation, and then to execute an appropriate response. On the other hand, the bird that had likely memorised their vocabulary did not use bigram frequency to guide categorisation, and perhaps as a reflection of behaviour, the population of NCL neurons in this bird did not seem to be encoding reward-related information. Thus, it seems that when there is no opportunity to categorise based on reward differences, NCL neurons seem to still associate rewards with category information during learning to guide decision making, but after learning do not need to continue to encode reward in this way (see Figure 8-1).

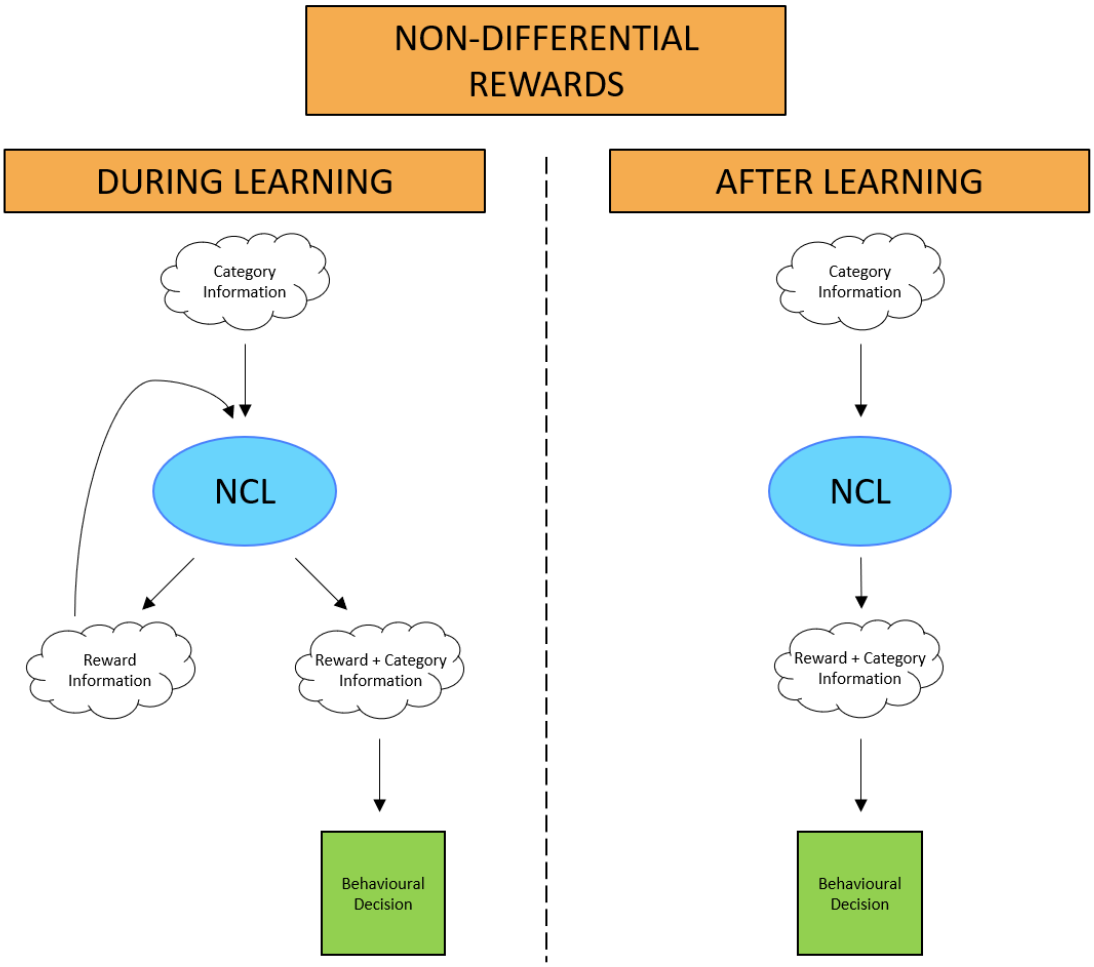

Figure 8-1: A model of NCL involvement in categorisation with non-differential rewards (Part 1).

Part 1: Using neural population data from our findings in Chapter 4, we model the potential role of NCL in categorisation, both during learning and after learning is achieved.

In our second categorisation study, we found significant hemispheric differences in NCL neurons, and their involvement in categorisation. Left hemisphere NCL neurons were specialised for encoding information about differences in reward (i.e. reward versus no reward), and then using that information to execute a response. On the other hand, right hemisphere NCL did not appear to be involved in these processes. It is important to note that the S+/S- discrimination used in our second study allowed birds to use reward

to categorise, whereas our first study did not. Therefore, while we find a significant hemispheric dominance in left NCL for reward-related processing, we can only conclude that this occurs when there is the opportunity to encode reward differences (see Figure 8-2). When there is no opportunity to encode reward differences, NCL must use other information, whether that be visual information or behavioural information, to distinguish between categories and execute the appropriate behavioural decision (Figure 8-1).

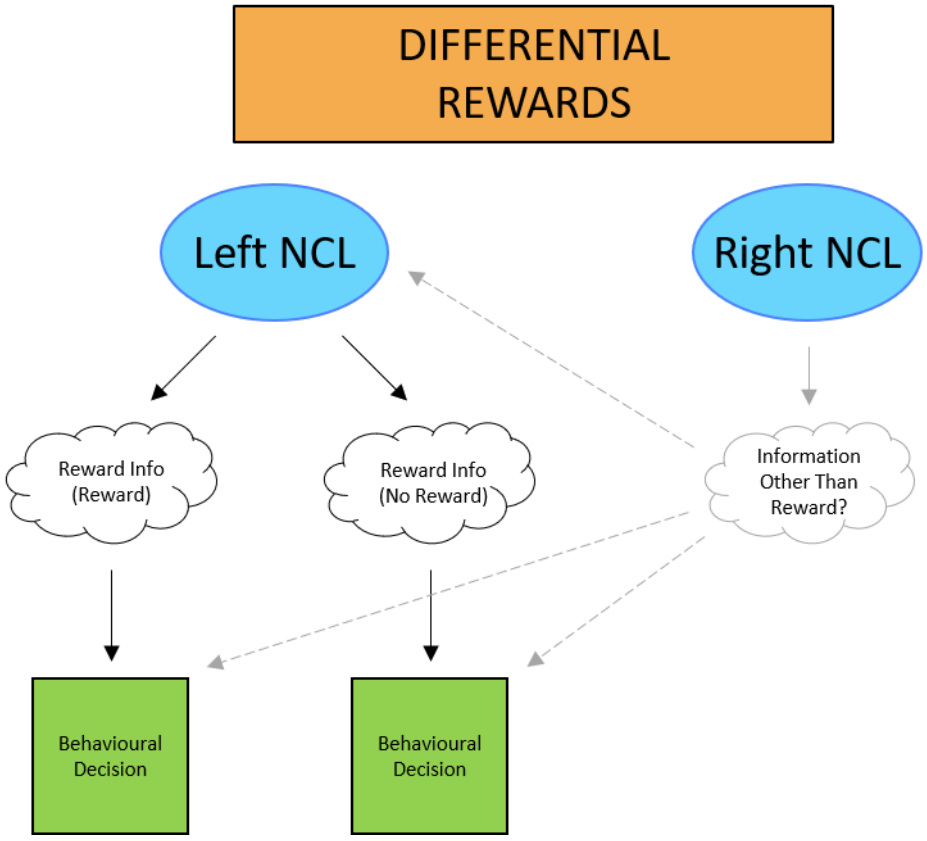

Figure 8-2: A model of NCL involvement in categorisation with differential rewards.

Using neural population data from our findings in Chapter 5, we model the potential role of NCL in categorisation, for both hemispheres. Grey indicates speculation beyond our findings.

In our third categorisation study, we found differences in the way excitatory and inhibitory NCL neurons are involved in the categorisation process. Inhibitory neurons, which make up the majority of NCL neurons we recorded from, are involved in executing differential behavioural responses (as these neurons are most active during the choice period). Furthermore, at the single cell level, in which the majority are inhibitory, we again found significant differences in firing rates associated with colour differences in relevant stimuli at the point when a decision is required. On the other hand, the smaller population of excitatory NCL neurons appear to be encoding differences in visual features (colour) between behaviourally relevant and irrelevant stimuli. We posit that the visual-behavioural information encoded by excitatory neurons is then passed onto inhibitory neurons to execute a behavioural decision (see Figure 8-3). We will discuss where the visual information that excitatory neurons associate with behavioural relevancy may come from in section 8.2.4.

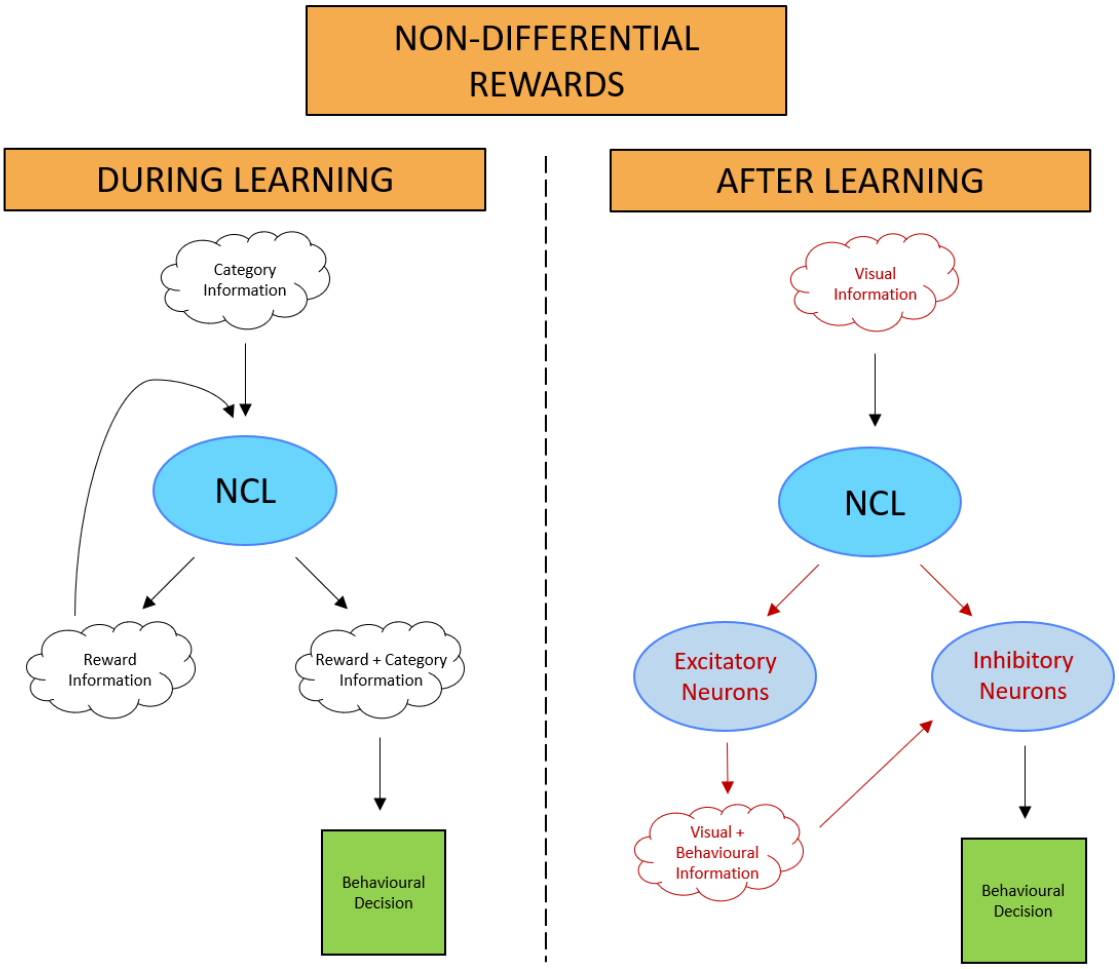

Figure 8-3: A model of NCL involvement in categorisation with non-differential rewards (Part 2).

Part 2: Using neural population data from our findings in Chapter 6, we add to the previous model of potential role of NCL in categorisation, for both excitatory and inhibitory neurons. Red indicates changes to the previous model.

In our final categorisation study, the fact that we found more delay-sensitive neurons over visually responsive neurons reinforces the idea that NCL neurons are highly involved in preparing to execute behaviour. However, we did find that some NCL neurons were visually responsive, and it was only in these neurons that we found category effects. Like our third study, among these visually responsive neurons, we found that excitatory

neurons appear to be encoding visual differences in the stimuli. Although we did not have enough neural data to make robust conclusions, we also expect that like our third study, these excitatory neurons are associating visual information with behaviourally relevant information. Furthermore, we found some evidence that inhibitory NCL neurons were encoding reward differentially based on which category the reward was associated with, despite there being no difference in reward. However, we leave our model of NCL involvement as it stands as the data gathered from our final study is limited.

8.2.2. The role of ENTO in categorisation

Based on previous literature discussed in Chapter 1, we expected ENTO to be primarily involved in using visual information, that is, the visual properties of stimuli, to discriminate between categories. In our first categorisation study, we did not find any evidence of ENTO neurons showing differential activity between words and nonwords. We argue that the lack of differential activity was likely caused by an inability to properly measure the period in which the birds were looking at the four-letter string. However, we believe that ENTO neurons use both visual and behavioural information to form categories during learning, but after learning, ENTO neurons primarily encode visual differences in categories (see Figure 8-4).

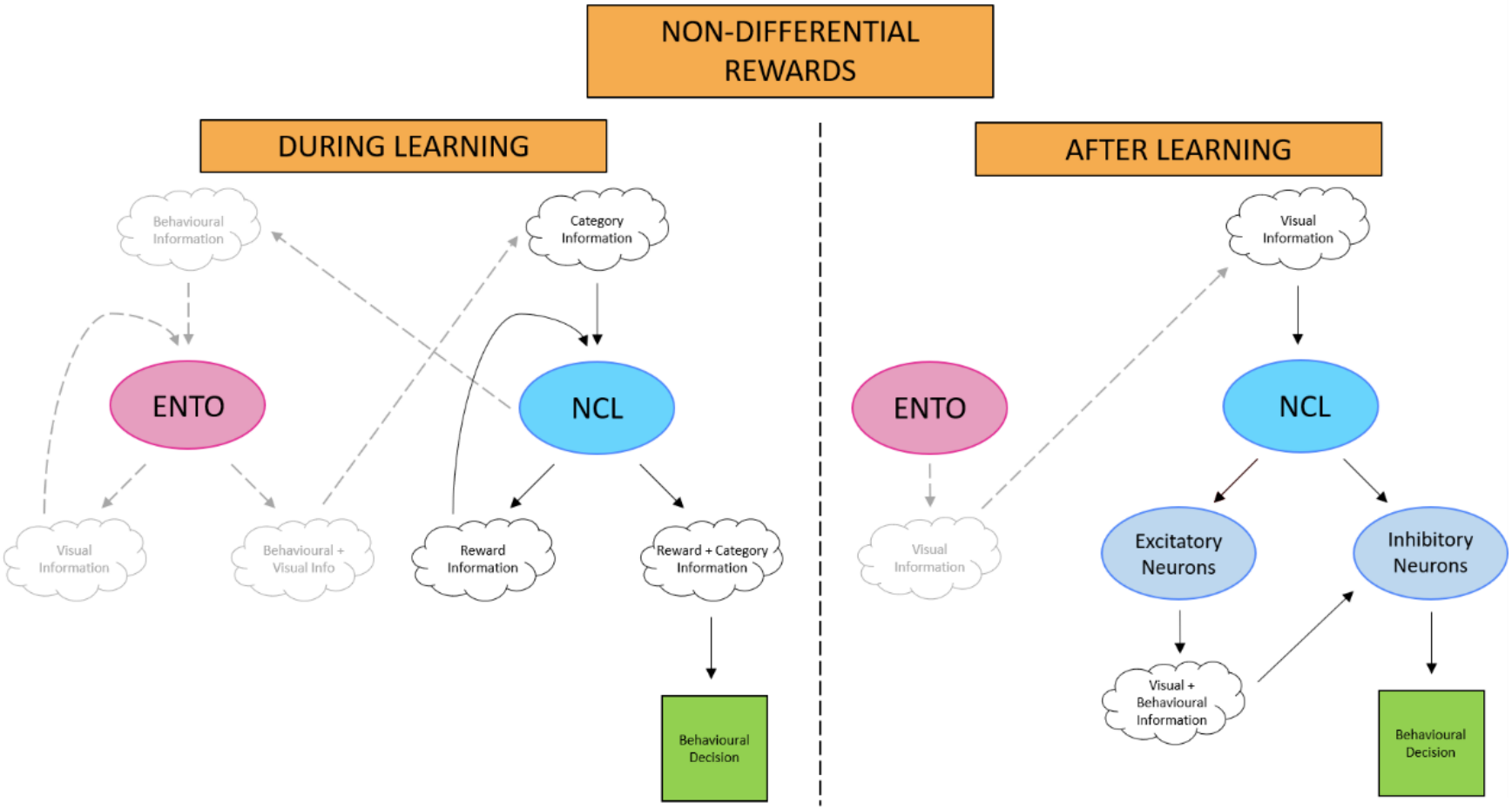

Figure 8-4: A model of ENTO and NCL involvement in categorisation with non-differential rewards (Part 1).

Part 1: Using neural population data from our findings in Chapter 4, we model the potential role of ENTO and NCL in categorisation, both during and after learning. Grey indicates speculation beyond our findings.

In our second study, we found significant hemispheric differences in ENTO neurons and their involvement in categorisation. Left hemisphere ENTO neurons were specialised for processing local visual features of the stimuli to discriminate between categories, but also appear to incorporate reward information to help form the categories. Thus, while ENTO neurons do seem to primarily categorise based on differences in visual features, these neurons also incorporate reward information to aid categorisation when there is the opportunity to do so (i.e., when the task results in differential reward). On the other hand, right hemisphere ENTO neurons did not seem to be involved in distinguishing between categories based on visual features, nor did right hemisphere neurons incorporate information about reward. We argue that while left NCL neurons primarily encode reward information, and left ENTO neurons primarily encode visual information, both of these areas must be sharing the information with one another to aid the categorisation process (see Figure 8-5).

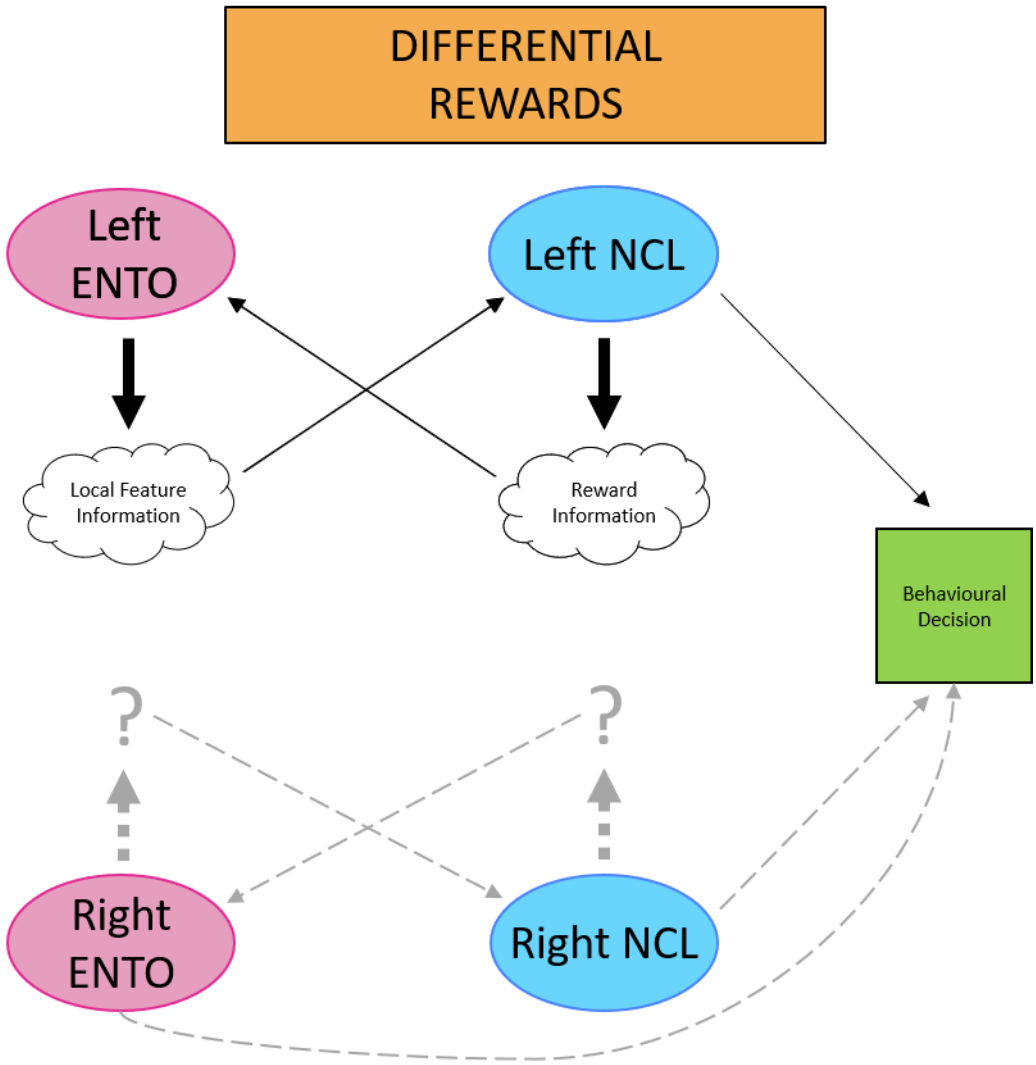

Figure 8-5: A model of ENTO and NCL involvement in categorisation with differential rewards.

Using neural population data from our findings in Chapter 5, we model the potential role of ENTO and NCL in categorisation, for both hemispheres. Grey indicates speculation beyond our findings.

In our third categorisation study, we also failed to find evidence of ENTO neurons encoding differences in visual features at the population level, including visual differences in behaviourally relevant versus irrelevant stimuli. However, we believe that

ENTO neurons are encoding differences in visual features, but that the way in which we have analysed our data is masking the results (see Chapter 6 for a more detailed explanation). Indeed, when we analysed our data at the single neuron level, we found significant differences in firing rates associated with colour differences in relevant stimuli at the point when the bird is looking at the stimuli, with relatively equal numbers of excitatory and inhibitory neurons. Therefore, we theorise that under non-differential reward conditions, both excitatory and inhibitory ENTO neurons process differences in local features, and then relay that visual information onto excitatory NCL neurons to aid decision making (see Figure 8-6). Unfortunately, we were not able to analyse activity to individual stimuli at the single cell level, but we would have expected to also find visual differences here as our other results indicate that ENTO neurons are encoding local features.

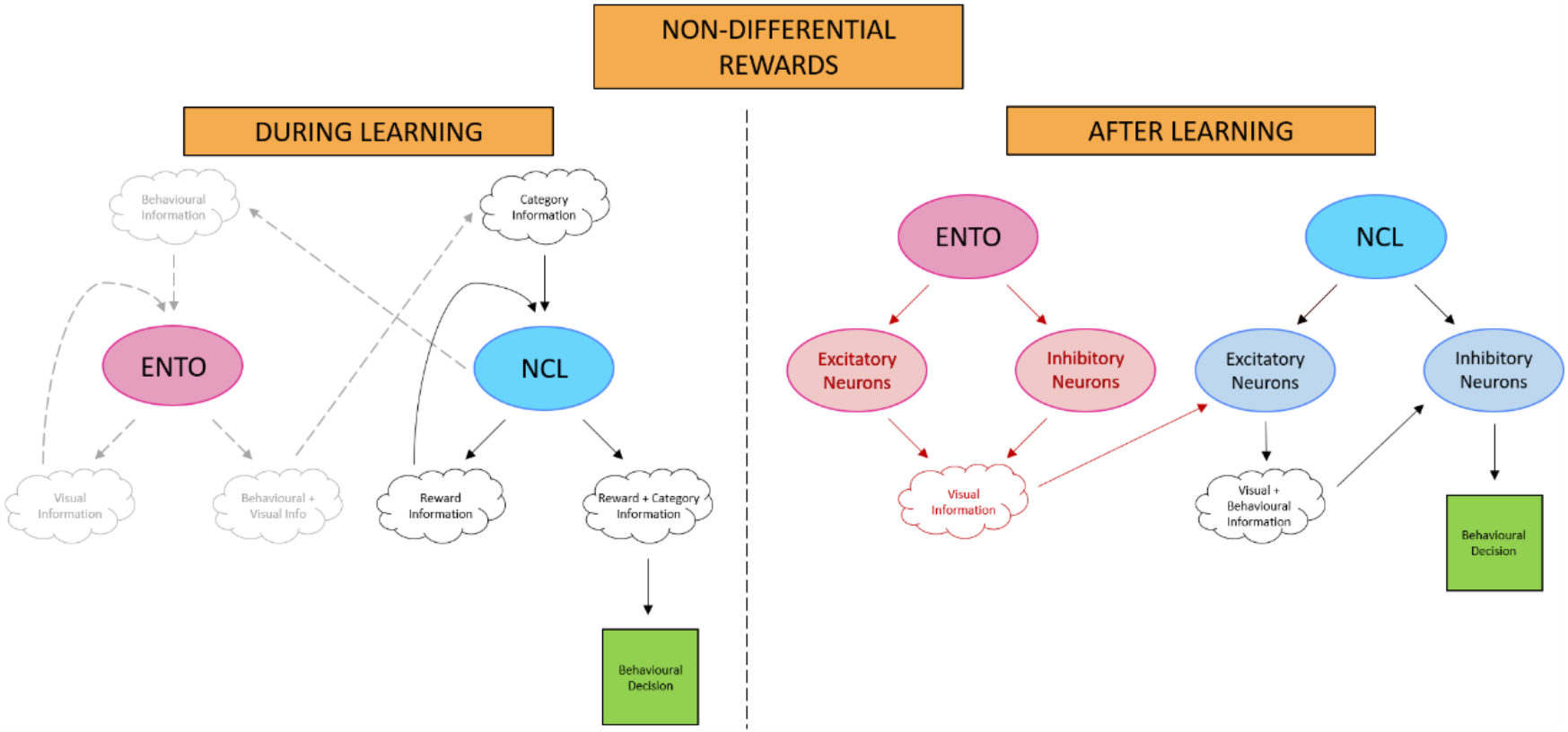

Figure 8-6: A model of ENTO and NCL involvement in categorisation with non-differential rewards (Part 2).

Part 2: Using neural population data from our findings in Chapter 6, we model the potential role of ENTO and NCL in categorisation, for both excitatory and inhibitory neurons. Red indicates changes to the previous model., and grey indicates speculation beyond our findings.

In our final categorisation study, the fact that we found equal numbers of neurons that were visually responsive and delay sensitive, which provides further evidence that ENTO is involved in visual feature discrimination, but also shares visual and behavioural information with NCL. We did not find any category effects within visually responsive ENTO neurons as expected. However, a lack of evidence is likely due to the small sample size, and thus we leave our model of ENTO and NCL involvement as it stands.

8.2.3. The role of MVL in categorisation

Although we only recorded from MVL in two of our four categorisation studies, we will postulate MVL's involvement in the categorisation process alongside ENTO and NCL. Our first set of MVL data was recorded during our second categorisation study. We found that both the left and right hemisphere encoded differences in visual features between categories. Previous literature has shown that the left hemisphere is specialised for detecting and discriminating local features, while the right hemisphere is specialised for discriminating based on global features (Yamazaki et al., 2007). While the intermediary visual area (ENTO) appears to focus on encoding local features, we present evidence that one of the higher-order visual areas (MVL) encodes both local and global features, and likely incorporates this information to guide categorisation. It is likely that the incorporated visual information is then relayed to NCL to execute a decision (Figure 8-7), either directly via reciprocal projections with NCL (Atoji & Wild, 2012), or indirectly via the reciprocal connections between ENTO and MVL (Krutzfeldt & Wild, 2005).

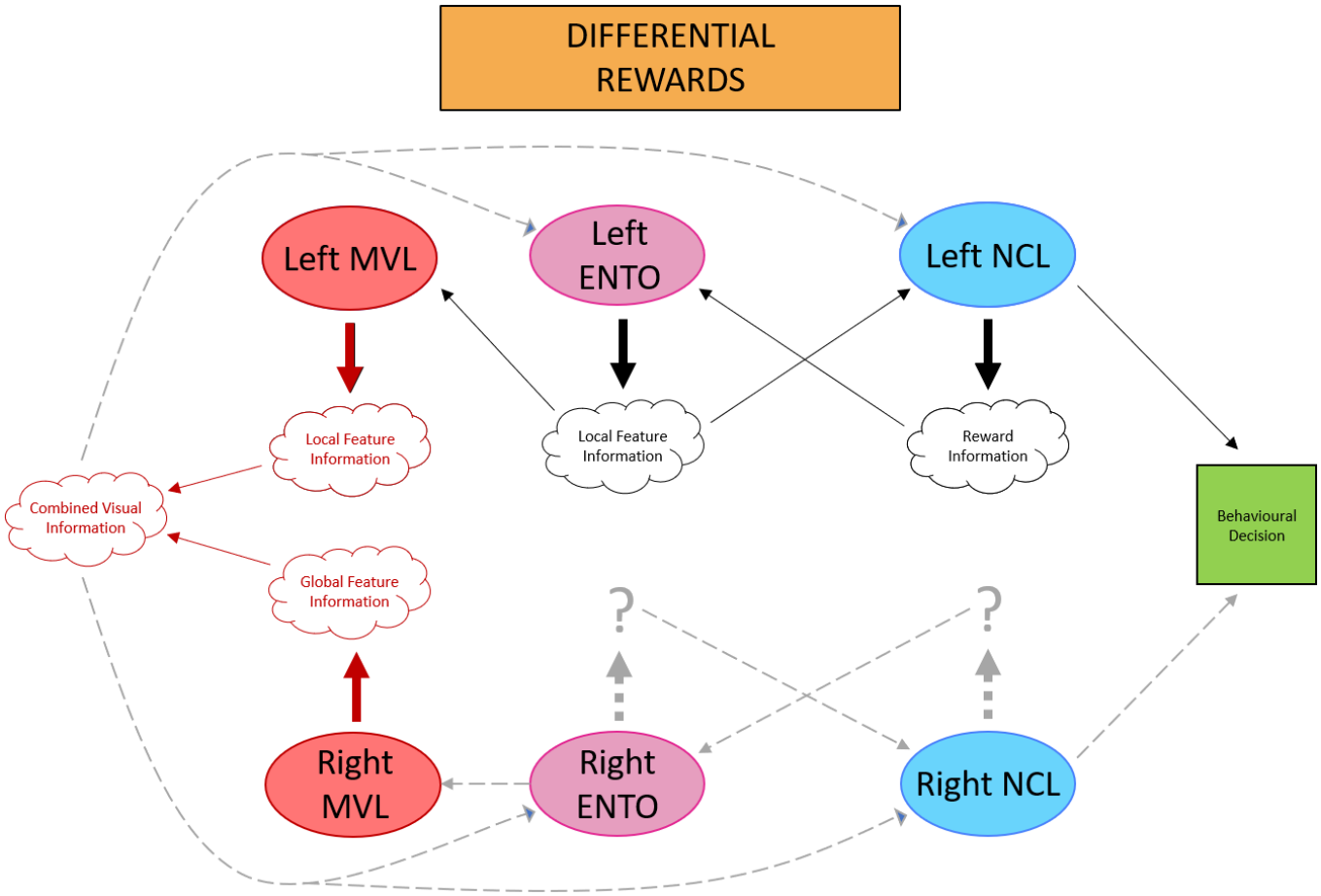

Figure 8-7: A model of MVL, ENTO, and NCL involvement in categorisation with differential rewards.

Using neural population data from our findings in Chapter 5 to model the potential role of MVL, ENTO, and NCL in categorisation, for both hemispheres. Red indicates changes to the previous model, and grey indicates speculation beyond our findings.

Our second set of MVL data was recorded during our final categorisation task. We found more visually responsive neurons than delay sensitive neurons in MVL, which we believe is reflective of MVL being involved with encoding visual information during categorisation. Like in ENTO, we did not find any category effects within visually responsive neurons, although the lack of evidence is likely again due to the small number of neurons recorded from.

8.3. Future Directions

We have presented our theorised model of the neural mechanisms of categorisation across NCL and ENTO in the pigeon brain, for tasks with and without differential rewards. However, we do not presume that our model provides a complete overview of the way these areas are involved in categorisation. For example, we were unable to determine what role right hemisphere NCL and ENTO play in the categorisation process under differential rewards. While it may be possible that the right hemispheres of these areas are not involved in categorical processes, we cannot say for certain whether that is the case. We also theorise how these areas interact with one another during categorisation, and what information is relayed between them. The connections described between these areas is grounded in histological and anatomical literature that shows that these areas project to one another, but it is difficult to ascertain whether these pathways are used during categorisation specifically. In both of these cases, it is the limitations of single-unit electrophysiology that prevent us from fully understanding the processes within and between these areas.

Firstly, single-unit electrophysiology is limited in that we can only record from single neurons, rather than recording across multiple neurons simultaneously. While we can compile data from single neurons to form a representation of the population, it would be

preferable to record from multiple neurons simultaneously to more thoroughly understand how neurons in each area work together during categorisation. Secondly, in most cases, we cannot record from multiple areas or hemispheres in the same bird, and thus cannot account for individual differences. While we hope that normalisation and combining the data to form population profiles would remove most of the variance between birds, it is always possible that individual variances still exist. In order to ameliorate these limitations, other categorisation studies using multiple-channel electrophysiology could help elucidate each area's role in the categorisation process.

Outside of the general limitations associated with single-unit electrophysiology, further research into the neural correlates of categorisation is necessary to fill the gaps in our model. We adapted our model as we progressed through all four of our studies, each of which improved on the paradigm of the previous study. We believe that our final study, the DMC paradigm, is the best task to help detangle the neural correlates of categorisation in pigeons. Unfortunately, due to time restrictions, we were unable to record from more than one bird, in just one hemisphere. Thus, we believe that a larger sample of data from such a task would help to either reinforce our model as it currently stands, or even potentially reveal different mechanisms of categorisation in these areas. The DMC paradigm could also be adapted to have differential reward outcomes, and further comparisons between the role of each area when differential and non-differential rewards are available could be made. As we found significant hemispheric differences in our second study, we also suggest comparing data from each hemisphere. We have also posited that each area encodes information about categories differently when learning categories versus discriminating learned categories. While we did not specifically aim to

investigate neural activity during learning, making such a comparison would be interesting and useful in unpacking each area's role in the categorisation process.

Finally, although it was not the main scope of this thesis, we have provided some preliminary insights into the role of a higher-order visual area (MVL) during categorisation. At the start of this thesis project, ENTO was widely considered as the avian analogue of primate IT cortex. However, as the project progressed, more and more evidence has indicated that ENTO is just an intermediary area similar to extrastriate cortex, while other higher-order areas like MVL, NFL, TPO, and NIL are more likely to be analogues of IT cortex. Therefore, in order to round out our understanding of the role visual areas play in categorisation, more data from these higher-order areas is needed. Previous literature has also not fully investigated the role of the other intermediary visual area, the Wulst, in these processes. Furthermore, we do not claim that only visual and prefrontal areas like NCL would be involved in categorisation; other areas like the hippocampus are also likely to be a part of the pathway we have posited in our models. A more complete understanding of how the neurons in the pigeon brain encode and process categorical information requires investigation across the whole brain. Categorisation is a fundamental cognitive process across a wide range of species and understanding the mechanisms behind categorisation in a visual animal such as the pigeon may help illuminate underlying common mechanisms in humans and other mammals.

REFERENCES

Ahumada-Galleguillos, P., Fernández, M., Marin, G. J., Letelier, J. C., & Mpodozis, J. Anatomical organization of the visual dorsal ventricular ridge in the chick (*Gallus gallus*): Layers and columns in the avian pallium. *Journal of Comparative Neurology*, *523*(17), 2618-2636. https://doi.org/10.1002/cne.23808

Allman, J. M., & Kaas, J. H. (1971). Representation of the visual field in striate and adjoining cortex of the owl monkey (*Aotus trivirgatus*). *Brain Research*, *35*, 89-106. https://doi.org/10.1016/0006-8993(71)90596-8

Alonso, Y. (1998). Lateralization of visually guided behaviour during feeding in zebra finches (*Taeniopygia guttata*). *Behavioural Processes*, *43*(3), 257-263. https://doi.org/10.1016/S0376-6357(98)00015-1

Anderson, C., & Colombo, M. (2019). Matching-to-sample: Comparative overview. In J. Vonk & T. Shackelford (Eds.), *Encyclopedia of Animal Cognition and Behavior*. Springer International Publishing.

Anderson, C., Johnston, M., Marrs, E. J., Porter, B., & Colombo, M. (2020). Delay activity in the Wulst of pigeons (*Columba livia*) represents correlates of both sample and reward information. *Neurobiology of Learning and Memory*, *171*, 107214. https://doi.org/10.1016/j.nlm.2020.107214

Anderson, C., Parra, R. S., Chapman, H., Steinemer, A., Porter, B., & Colombo, M. (2020). Pigeon nidopallium caudolaterale, entopallium, and mesopallium

ventrolaterale neural responses during categorisation of Monet and Picasso paintings. *Scientific Reports*, *10*, 15971. https://doi.org/10.1038/s41598-020-72650-y

Ashby, F. G., & Ell, S. W. (2001). The neurobiology of human category learning. *Trends in Cognitive Sciences*, *5*(5), 204-210. https://doi.org/10.1016/S1364-6613(00)01624-7

Ashby, F. G., & Maddox, W. T. (2011). Human category learning 2.0. *Annals of the New York Academy of Sciences*, *1224*, 147-161. https://doi.org/10.1111/j.1749-6632.2010.05874.x

Atoji, Y., Sarkar, S., & Wild, J. M. (2018). Differential projections of the densocellular and intermediate parts of the hyperpallium in the pigeon (*Columba livia*). *Journal of Comparative Neurology*, *526*(1), 146-165. https://doi.org/10.1002/cne.24328

Atoji, Y., & Wild, J. M. (2012). Afferent and efferent projections of the mesopallium in the pigeon (*Columba livia*). *The Journal of Comparative Neurology*, *520*(4), 717-741. https://doi.org/10.1002/cne.22763

Aust, U., & Huber, L. (2001). The role of item- and category-specific information in the discrimination of people versus nonpeople images by pigeons. *Animal Learning & Behaviour*, *29*(2), 107-119. https://doi.org/10.3758/BF03192820

Aust, U., & Huber, L. (2002). Target-defining features in a "people-present/people-absent' discrimination by pigeons. *Animal Learning and Behavior*, *30*(2), 165-176. https://doi.org/10.3758/bf03192918

Awh, E., Armstrong, K. M., & Moore, T. (2006). Visual and oculomotor selection: Links, causes and implications for spatial attention. *Trends in Cognitive Sciences*, *10*(3), 124-130. https://doi.org/10.1016/j.tics.2006.01.001

Azizi, A. H., Pusch, R., Koenen, C., Klatt, S., Bröker, F., Thiele, S., Kellermann, J., Güntürkün, O., & Cheng, S. (2019). Emerging category representation in the visual forebrain hierarchy of pigeons (*Columba livia*). *Behavioural Brain Research*, *356*, 423-434. https://doi.org/10.1016/j.bbr.2018.05.014

Berg, M. E., & Grace, R. C. (2011). Categorisation of multidimensional stimuli by pigeons. *Journal of the Experimental Analysis of Behaviour*, *95*(3), 305-326. https://doi.org/10.1901/jeab.2010.94-305

Bessette, B. B., & Hodos, W. (1989). Intensity, color, and pattern discrimination deficits after lesions of the core and belt regions of the ectostriatum. *Visual Neuroscience*, *2*, 27-34. https://doi.org/10.1017/S0952523800004296

Bhatt, R. S., Wasserman, E. A., Reynolds, W. F. Jr., & Knauss, K. S. (1988). Conceptual behaviour in pigeons: Categorisation of both familiar and novel examples from four classes of natural and artificial stimuli. *Journal of Experimental Psychology: Animal Behavior Processes*, *14*(3), 219-234. https://doi.org/10.1037//0097-7403.14.3.219

Bilkey, D. K., Russell, N., & Colombo, M. (2003). A lightweight microdrive for single-unit recording in freely moving rats and pigeons. *Methods*, *30*(2), 152–158. https://doi.org/10.1016/S1046-2023(03)00076-8

Blair, M. R., Watson, M. R., Walshe, C. R., & Maj, F. (2009). Extremely selective attention: Eye-tracking studies of the dynamic allocation of attention to stimulus features in categorisation. *Journal of Experimental Psychology*, *35*(5), 1196-1206. https://doi.org/10.1037/a0016272

Blough, D. S. (1982). Pigeon perception of letters of the alphabet. *Science*, *218*(4570), 397-398. https://doi.org/10.1126/science.7123242

Bosse, M. (2015). Learning to read and spell: How children acquire word orthographic knowledge. *Child Development Perspectives*, *9*(4), 222-226. https://doi.org/10.1111/cdep.12133

Bosse, M., Chaves, N., Largy, P., & Valdois, S. (2013). Orthographic learning during reading: the role of whole-word visual processing. *Journal of Research in Reading*, *38*(2), 141-158. https://doi.org/10.1111/j.1467-9817.2012.01551.x

Broadbent, D. E., & Gregory, M. (1968). Visual perception of words differing in letter diagram frequency. *Journal of Verbal Learning and Verbal Behavior*, *7*(2), 569-571. https://doi.org/10.1016/S0022-5371(68)80052-0

Broschard, M. B., Kim, J., Love, B. C., Wasserman, E. A., & Freeman, J. H. (2019). Selective attention in rat visual category learning. *Learning and Memory*, *26*, 84-92. https://doi.org/10.1101/lm.048942.118

Browning, R., Overmier, J. B., & Colombo, M. (2011). Delay activity in avian prefrontal cortex—sample code or reward code? *The European Journal of Neuroscience*, *33*(4), 726–735. https://doi.org/10.1111/j.1460-9568.2010.07540.x

Butter, C. M., & Gekoski, W. L. (1966). Alterations in pattern equivalence following inferotemporal and lateral striate lesions in rhesus monkeys. *Journal of Comparative and Physiological Psychology*, *61*(2), 309-312. https://doi.org/10.1037/h0023124

Castro, L., & Wasserman, E. A. (2014). Pigeons' tracking of relevant attributes in categorisation learning. *Experimental Psychology: Animal Learning and Cognition*, *40*(2), 195-211. https://psycnet.apa.org/doi/10.1037/xan0000022

Clark, W. J., & Colombo, M. (2020, in press). The functional architecture, receptive field characteristics, and representation of objects in the visual network of the pigeon brain. *Progress in Neurobiology*, 101781. https://doi.org/10.1016/j.pneurobio.2020.101781

Clark, W. J., Porter, B., & Colombo, M. (2019). Searching for face-category representation in the avian visual forebrain. *Frontiers in Physiology*, *10*, 140. https://doi.org/10.3389/fphys.2019.00140

Clayton, N. S., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature*, *395*(6699), 272-274. https://doi.org/10.1038/26216

Clayton, N. S., & Krebs, J. R. (1994). Memory for spatial and object-specific cues in food-storing and non-storing birds. *Journal of Comparative Physiology A*, *174*, 371-379. https://doi.org/10.1007/BF00240218

Colombo, M., Frost, N., & Steedman, W. (2001). Responses of ectostriatal neurons during delayed matching-to-sample behavior in pigeons (*Columba livia*). *Brain Research*, *917*(1), 55–66. https://doi.org/10.1016/S0006-8993(01)02906-7

Colombo, M., & Scarf, D. (2020). Are there differences in "intelligence" between nonhuman species? The role of contextual variables. *Frontiers in Psychology*, *11*, 2072. https://doi.org/10.3389/fpsyg.2020.02072

Cook, R. G. (2000). The comparative psychology of avian visual cognition. *Current Directions in Psychological Science*, *9*(3), 83-89. https://doi.org/10.1111/1467-8721.00066

Cook, R. G., Levison, D. G., Gillett, S. R., & Blaisdell, A. P. (2005). Capacity and limits of associative memory in pigeons. *Psychonomic Bulletin & Review, 12*(2), 350-358. https://doi.org/10.3758/bf03196384

Cook, R. G., Wright, A. A., & Drachman, E. E. (2013). Categorisation of birds, mammals, and chimeras by pigeons. *Behavioural Processes, 93*, 98-110. https://doi.org/10.1016/j.beproc.2012.11.006

Cowey, A. (1964). Projection of the retina onto striate and prestriate cortex in the squirrel monkey, *Saimiri sciureus*. *Journal of Neurophysiology, 27*, 366-393. https://doi.org/10.1152/jn.1964.27.3.366

Cowey, A., & Weiskrantz, L. (1967). A comparison of the effects of inferotemporal and striate cortex lesions on the visual behavior of rhesus monkeys. *Quarterly Journal of Experimental Psychology*, *19*, 246-253. https://doi.org/10.1080/14640746708400099

Cromer, J. A., Roy, J. E., & Miller, E. K. (2010). Representation of multiple, independent categories in the primate prefrontal cortex. *Neuron, 66*(5), 796-807. https://doi.org/10.1016/j.neuron.2010.05.005

Curtis, C. E., & D'Esposito, M. (2004). The effects of prefrontal lesions on working memory performance and theory. *Cognitive, Affective, & Behavioral Neuroscience, 4*(4), 528-539. https://doi.org/10.3758/CABN.4.4.528

D'Amato, M. R., & Van Sant, P. (1988). The person concept in monkeys (*Cebus apella*). *Journal of Experimental Psychology: Animal Behavior Processes*, *14*(1), 43-55. https://doi.org/10.1037/0097-7403.14.1.43

Daisley, J. N., Mascalzoni, E., Rosa-Salva, O., Rugani, R., & Regolin, L. (2009). Lateralization of social cognition in the domestic chicken (*Gallus gallus*). *Philosophical Transactions of the Royal Society B, 364*, 965-981. https://doi.org/10.1098/rstb.2008.0229

Desimone, R. (1996). Neural mechanisms for visual memory and their role in attention. *Proceedings of the National Academy of Sciences USA, 93*(24), 13494-13499. https://doi.org/10.1073/pnas.93.24.13494

Diekamp, B., Gagliardo, A., & Güntürkün, O. (2002). Nonspatial and subdivision-specific working memory deficits after selective lesions of the avian prefrontal cortex. *Journal of Neuroscience*, *22*(21), 9573-9580. https://doi.org/10.1523/JNEUROSCI.22-21-09573.2002

Dittrich, L., Rose, J., Buschmann, J-U. F., Bourdonnais, M., & Güntürkün, O. (2010). Peck tracking: A method for localising critical features within complex pictures for pigeons. *Animal Cognition, 13*(1), 133-143. https://doi.org/10.1007/s10071-009-0252-x

Ditz, H. M., & Nieder, A. (2015). Neurons selective to the number of visual items in the corvid songbird endbrain. *PNAS*, *112*(25), 7827-7832, https://doi.org/10.1073/pnas.1504245112

Ditz, H. M., & Nieder, A. (2016). Sensory and working memory representations of small and large numerosities in the crow endbrain. *The Journal of Neuroscience, 36*(47), 12044-12052. https://doi.org/10.1523/JNEUROSCI.1521-16.2016

Divac, I., & Mogensen, J. (1985). The prefrontal “cortex” in the pigeon catecholamine histofluorescence. *Neuroscience*, *15*(3), 677-682. https://doi.org/10.1016/0306-4522(85)90069-7

Duñabeitia, J. A., Orihuela, K., & Carreiras, M. (2014). Orthographic coding in illiterates and literates. *Psychological Science*, *25*(6), 1275-1280. https://doi.org/10.1177/0956797614531026

Dunnett, S. B., Nathwani, F., & Brasted, P. J. (1999). Medial prefrontal and neostriatal lesions lesions disrupt performance in an operant delayed alternation task in rats. *Behavioural Brain Research*¸ *106*(1-2), 13-28. https://doi.org/10.1016/S0166-4328(99)00076-5

Dykes, M., Klarer, A., Porter, B., Rose, J., & Colombo, M. (2018). Neurons in the pigeon nidopallium caudolaterale display value-related activity. *Scientific Reports*, *8*(1), 5377. https://doi.org/10.1038/s41598-018-23694-8

El-Nasan, A., Veeramachaneni, S., & Nagy, G. (2001). Word discrimination based on bigram co-occurences. In *Proceedings of Sixth International Conference on Document Analysis and Recognition* (pp. 149-153). Seattle, WA: IEEE. https://doi.org/10.1109/ICDAR.2001.953773

Fabre-Thorpe, M. (2003). Visual categorisation: Accessing abstraction in non-human primates. *Philosophical Transactions of the Royal Society B*, *358*(1435), 1215-1223. https://doi.org/10.1098/rstb.2003.1310

Fabre-Thorpe, M., Richard, G., & Thorpe, S. J. (1998). Rapid categorisation of natural images by rhesus monkeys. *NeuroReport*, *9*(2), 303-308.

Freedman, D. J., Riesenhuber, M., Poggio, T., & Miller, E. K. (2001). Categorical representation of visual stimuli in the primate prefrontal cortex. *Science*, *291*(5502), 312-216. https://doi.org/10.1126/science.291.5502.312

Freedman, D. J., Riesenhuber, M., Poggio, T., & Miller, E. K. (2003). A comparison of primate prefrontal and inferior temporal cortices during visual categorisation. *Journal of Neuroscience*, *23*(12), 5235-5246. https://doi.org/10.1523/JNEUROSCI.23-12-05235.2003

Gagliardo, A., Bonadonna, F., & Divac, I. (1996). Behavioural effects of ablations of the presumed 'prefrontal cortex' or the corticoid in pigeons. *Behavioural Brain Research*, *78*(2), 155-162. https://doi.org/10.1016/0166-4328(95)00243-x

Gattass, R., Gross, C. G., & Sandell, J. H. (1981). Visual topography of V2 in the macaque. *Journal of Comparative Neurology*, *201*, 519-539. https://doi.org/10.1002/cne.902010405

Ghosh, N., Lea, S. E. G., & Noury, M. (2004). Transfer to intermediate forms following concept discrimination by pigeons: Chimeras and morphs. *Journal of the Experimental Analysis of Behavior*, *82*(2), 125-141. https://doi.org/10.1901/jeab.2004.82-125

Gibson, B. M., Wasserman, E. A., Gosselin, F., & Schyns, P. G. (2005). Applying Bubbles to localise features that control pigeons' visual discrimination behaviour. *Journal of Experimental Psychology*, *31*(3), 376-382. https://doi.org/10.1037/0097-7403.31.3.376

Gluck, M. A., Poldrack, R. A., & Kéri, S. (2008). The cognitive neuroscience of category learning. *Neuroscience and Biobehavioral Reviews*, *32*(2), 193-196. https://doi.org/10.1016/j.neubiorev.2007.11.002

Goodale, M. A. (1983). Visually guided pecking in the pigeon (*Columba livia*). *Brain, Behavior and Evolution*, *22*(1), 22–41. https://doi.org/10.1159/000121504

Grainger, J., Dufau, S., Montant, M., Ziegler, J. C., & Fagot, J. (2012). Orthographic processing in baboons (*Papio papio*). *Science*, *336*(6078), 245-248. https://doi.org/10.1126/science.1218152

Gross C. G. (1973). Visual functions of inferotemporal cortex. In R. Jung (Eds). Visual Centers in the Brain. *Handbook of Sensory Physiology (Central Processing of Visual Information Part B)*, vol 7, Part 3B (pp. 451-482). Berlin: Springer-Verlag.

Gross, C. G., Rocha-Miranda, C. E., & Bender, D. B. (1972). Visual properties of neurons in inferotemporal cortex of the macaque. *Journal of Neurophysiology*, *35*(1), 96-111. https://doi.org/10.1152/jn.1972.35.1.96

Gross, C. G., Rodman, H. R., Gochin, P. M., & Colombo, M. (1993). Inferior temporal cortex as a pattern recognition device. In E. Baum (Ed.). *Proceedings of the Third Annual NEC Research Symposium*, (pp. 44-73). Philadelphia: Siam Press.

Gu, Y., Wang, Y., Zhang, T., & Wang, S. (2002). Stimulus size selectivity and receptive field organization of ectostriatal neurons in the pigeon. *Journal of Comparative Physiology*, *188*(3), 173-178. https://doi.org/10.1007/s00359-002-0290-1

Güntürkün, O. (1997). Cognitive impairments after lesions of the neostriatum caudolaterale and its thalamic afferent: functional similarities to the mammalian prefrontal system?. *Journal Fur Hirnforschung*, *38*(1), 133-143.

Güntürkün, O. (2005a). Avian and mammalian “prefrontal cortices”: Limited degrees of freedom in the evolution of the neural mechanisms of goal-state maintenance. *Brain Research Bulletin*, *66*(4), 311–316. https://doi.org/10.1016/j.brainresbull.2005.02.004

Güntürkün, O. (2005b). The avian ‘prefrontal cortex’ and cognition. *Current Opinion in Neurobiology*, *15*(6), 686–693. https://doi.org/10.1016/j.conb.2005.10.003

Güntürkün, O., Diekamp, B., Manns, M., Nottelmann, F., Prior, H., Schwarz, A., & Skiba, M. (2000). Asymmetry pays: Visual lateralization improves discrimination success in pigeons. *Current Biology*, *10*(17), 1079-1081. https://doi.org/10.1016/S0960-9822(00)00671-0

Güntürkün, O., & Kesch, S. (1987). Visual lateralization during feeding in pigeons. *Behavioural Neuroscience*, *101*(3), 433-435. https://doi.org/10.1037/0735-7044.101.3.433

Gusel’nikov, V. I., Morenkov, E. D., & Hunh, D. C. (1977). Responses and properties of receptive fields of neurons in the visual projection zone of the pigeon hyperstriatum. *Neuroscience and Behavioral Physiology*, *8*(3), 210-215. https://doi.org/10.1007/BF01184060

Hannagan, T., Ziegler, J. C., Dufau, S., Fagot, J., Grainger, J. (2014). Deep learning of orthographic representations in baboons. *PLoS One*, *9*(1), e84843. https://doi.org/10.1371/journal.pone.0084843

Herrnstein, R. J. (1979). Acquisition, generalization, and discrimination reversal of a natural concept. *Journal of Experimental Psychology: Animal Behaviour Processes*, *5*(2), 116-129. https://doi.org/10.1037//0097-7403.5.2.116

Herrnstein, R. J. (1984). Objects, categories, and discriminative stimuli. In H. T. Roitblat, G. Bever, and H. S. Terrace (Eds.), *Animal Cognition*, (pp. 233-262). New Jersey: Lawrence Erlbaum Associates.

Herrnstein, R. J., & Loveland, D. H. (1964). Complex visual concept in the pigeon. *Science*, *146*(3643), 549-551. https://doi.org/10.1126/science.146.3643.549

Heywood, C. A., & Cowey, A. (1987). On the role of cortical area V4 in the discrimination of hue and pattern in macaque monkeys. *Journal of Neuroscience*, *7*(9), 2601-2617. https://doi.org/10.1523/JNEUROSCI.07-09-02601.1987

Hodos, W., Bessette, B. B., Macko, K. A., & Weiss, S. R. B. (1985). Normative data for pigeon vision. *Vision Research*, *25*(10), 1525-1527. https://doi.org/10.1016/0042-6989(85)90231-7

Hodos, W., & Karten, H. J. (1970). Visual intensity and pattern discrimination deficits after lesions of ectostriatum in pigeons. *Journal of Comparative Neurology*, *140*(1), 53-68. https://doi.org/10.1002/cne.901400104

Hodos, W., Weiss, S. R. B., & Bessette, B. B. (1988). Intensity difference thresholds after lesions of ectostriatum in pigeons. *Behavioural Brain Research*, *1*(1), 43-53. https://doi.org/10.1016/0166-4328(88)90007-1

Hulme, C., & Snowling, M. J. (2012). Learning to read: what we know and what we need to understand better. *Child Development Perspectives*, *7*(1), 1-5. https://doi.org/10.1111/cdep.12005

Izquierdo, A., Suda, R. K., & Murray, E. A. (2004). Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward

contingency. *The Journal of Neuroscience*, *24*(34), 7540-7548. https://doi.org/10.1523/JNEUROSCI.1921-04.2004

Jacobsen, C. F. (1936). Studies of cerebral function in primates. I. The functions of the frontal association areas in monkeys. *Comparative Psychology Monographs*, *13*(3), 1-60.

Jarvis, E. D., Güntürkün, O., Bruce, L., Csillag, A., Karten, H., Kuenzel, W., Medina, L., Paxinos, G., Perkel, D. J., Shimizu, T., Striedter, G., Wild, J. M., Ball, G. F., Dugas-Ford, J., Durand, S. E., Hough, G. E., Husband, S., Kubikova, L., Lee, D. W., Mello, C. V., Powers, A., Siang, C., Smulders, T. V., Wada, K., White, S. A., Yamamoto, K., Yu, J., Reiner, A., & Butler, A. B.; The Avian Brain Nomenclature Consortium [ABNC]. (2005). Avian brains and a new understanding of vertebrate brain evolution. *Nature Reviews Neuroscience*, *6*(2), 151-159. https://doi.org/10.1038/nrn1606

Jitsumori, M., & Yoshihara, M. (1997). Categorical discrimination of human facial expressions by pigeons: A test of the linear feature model. *The Quarterly Journal of Experimental Psychology*, *50*(3b), 253-268. https://doi.org/10/1080/713932657

Johnston, M., Anderson, C., & Colombo, M. (2017a). Neural correlates of sample-coding and reward-coding in the delay activity of neurons in the entopallium and nidopallium caudolaterale of pigeons (*Columba livia*). *Behavioural Brain Research*, *317*, 382–392. https://doi.org/10.1016/j.bbr.2016.10.003

Johnston, M., Anderson, C., & Colombo, M. (2017b). Pigeon NCL and NFL neuronal activity represents neural correlates of the sample. *Behavioral Neuroscience*, *131*(3), 213–219. https://doi.org/10.1037/bne0000198

Johnston, M., & Colombo, M. (2019). Entopallium. In J. Vonk & T. Shackelford (Eds.), *Encyclopedia of Animal Cognition and Behavior*. Springer International Publishing.

Karten, H., & Hodos, W. (1967). *A Stereotaxic Atlas of the Brain of the Pigeon (Columba livia)*. Baltimore: Johns Hopkins University Press.

Karten, H. J., Hodos, W., Nauta, W. J. H., & Revzin, A. M. (1973). Neural connections of the "visual wulst" of the avian telencephalon. Experimental studies in the pigeon (*Columba livia*) and owl (*Speotyto cunicularia*). *Journal of Comparative Neurology*, *150*(3), 253-278. https://doi.org/10.1002/cne.901500303

Keller, F. S., & Schoenfeld, W. N. (1950). *Principles of Psychology*. New York, NY: Appleton-Century-Crofts.

Keppel, G. (1982). *Design and Analysis: A Researcher's Handbook* (2nd ed.). Englewood Cliffs, NJ: Prentice-Hall, Inc.

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*, *8*(6), e1000412. https://doi.org/10.1371/journal.pbio.1000412

Kirsch, J. A., Vlachos, I., Hausmann, M., Rose, J., Yim, M. Y., Aertsen, A., & Güntürkün, O. (2009). Neuronal encoding of meaning: Establishing category-selective response patterns in the avian 'prefrontal cortex'. *Behavioural Brain Research*, *198*(1), 214-223. https://doi.org/10.1016/j.bbr.2008.11.010

Kling, J. W., & Riggs, L. A. (1971). *Woodworth and Schlossberg's Experimental Psychology* (3rd ed.). New York: Holt, Rinehart, and Winston.

Koenen, C., Millar, J., & Colombo, M. (2013). How bad do you want it? Reward modulation in the avian nidopallium caudolaterale. *Behavioral Neuroscience*, *127*(4), 544–554. https://doi.org/10.1037/a0033551

Koenen, C., Pusch, R., Bröker, F., Thiele, S., Güntürkün, O. (2016). Categories in the pigeon brain: A reverse engineering approach. *Journal of the Experimental Analysis of Behaviour*, *105*(1), 111-122. https://doi.org/10.1002/jeab.179

Kouwenhoven, M., & Colombo, M. (2016). The differential outcomes effect in pigeons (*Columba livia*): Is it truly anticipatory? *PloS One*, *11*(3), e0150510. https://doi.org/10.1371/journal.pone.0150510

Kriegeskorte, N., Mur, M., Ruff, D. A., Kiani, R., Bodurka, J., Esteky, H., Tanaka, K., & Bandettini, P. A. (2008). Matching categorical object representations in inferior temporal cortex of man and monkey. *Neuron*, *60*(60), 1126-1141. https://doi.org/10.1016/j.neuron.2008.10.043

Kröner, S., & Güntürkün, O. (1999). Afferent and efferent connections of the caudolateral neostriatum in the pigeon (*Columba livia*): A retro- and anterograde pathway tracing study. *Journal of Comparative Neurology*, *407*(2), 228-260. https://doi.org/10.1002/(sici)1096-9861(19990503)407:2<228::aid-cne6>3.0.co;2-2

Krützfeldt, N. O., & Wild, J. M. (2005). Definition and novel connections of the entopallium in the pigeon (*Columba livia*). *Journal of Comparative Neurology*, *490*(1), 40-56. https://doi.org/10.1002/cne.20627

Lawrence, D. H. (1949). Acquired distinctiveness of cues: I. Transfer between discrimination on the basis of familiarity with the stimulus. *Journal of Experimental Psychology*, *39*(6), 770-784. https://doi.org/10.1037/h0058097

Lea, S. E. G. (1984). In what sense do pigeons learn concepts?. In H. L. Roitblat, T. G. Bever, and H. S. Terrace (Eds.), *Animal Cognition*, (pp. 263-276). Hillsdale, NJ: Erlbaum.

Lengersdorf, D., Pusch, R., Güntürkün, O., & Stüttgen, M. C. Neurons in the pigeon nidopallium caudolaterale signal the selection and execution of perceptual decisions. *European Journal of Neuroscience*, *40*(9), 3316-3327. https://doi.org/10.1111/ejn.12698

Leutgeb, S., Husband, S., Riters, L. V., Shimizu, T., & Bingman, V. P. (1996). Telencephalic afferents to the caudolateral neostriatum of the pigeon. *Brain Research*, *730*(1-2), 173-181. https://doi.org/10.1016/0006-8993(96)00444-1

Li, D.-P., Xiao, Q., & Wang, S.-R. (2007). Feedforward construction of the receptive field and orientation selectivity of visual neurons in the pigeon. *Cerebral Cortex*, *17*(4), 885–893. https://doi.org/10.1093/cercor/bhk043

Liu, X., Wan, H., Li, S., Shang, Z., & Shi, L. (2017). The role of nidopallium caudolaterale in the goal-directed behaviour of pigeons. *Behavioural Brain Research*, *326*, 112-120. https://doi.org/10.1016/j.bbr.2017.02.042

Manns, M., & Ströckens, F. (2014). Functional and structural comparison of visual lateralization in birds – similar but still different. *Frontiers in Psychology*, *5*, 206. https://doi.org/10.3389/fpsyg.2014.00206

Mercado, E., Orduña, I., & Nowak, J. M. (2005). Auditory categorisation of complex sounds by rats (*Rattus norvegicus*). *Journal of Comparative Psychology*, *119*(1), 90-98. https://doi.org/10.1037/0735-7036.119.1.90

Miceli, D., Gioanni, H., Reperant, J., & Peyrichoux, J. (1979). The avian visual Wulst: I. An anatomical study of afferent and efferent pathways. II. An electrophysiological study of the functional properties of single neurons. In A. M. Granda and J. H. Maxwell (Eds.), *Neural Mechanisms of Behavior in the Pigeon*, (pp. 223-254). New York: Plenum Press.

Miller, E. K., Erickson, C. A., & Desimone, R. (1996). Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *The Journal of Neuroscience, 16*(16), 5154-5167. https://doi.org/10.1523/JNEUROSCI.16-16-05154.1996

Mohri, M., Riley, M., Hindle, D., Ljolje, A., & Pereira, F. (1998). Full expansion of context-dependent networks in large vocabulary speech recognition. In *Proceedings of the 1998 IEEE International Conference on Acoustics, Speech and Signal Processing*, (pp. 665-668). Seattle, WA: IEEE. https://doi.org/10.1109/ICASSP.1998.675352

Morgan, M. J., Fitch, M. D., Holman, M. D., & Lea, S. E. G. (1976). Pigeons learn the concept of an 'A'. *Perception*, *5*(1), 57-66. https://doi.org/10.1068/p050057

Ng, B. S. W., Grabska-Barwińska, A., Güntürkün, O., & Jancke, D. (2010). Dominant vertical orientation processing without clustered maps: early visual brain dynamics imaged with voltage-sensitive dye in the pigeon visual Wulst. *The Journal of Neuroscience, 30*(19), 6713–6725. https://doi.org/10.1523/JNEUROSCI.4078-09.2010

Nguyen, A. P., Spetch, M. L., Crowder, N. A., Winship, I. R., Hurd, P. L., & Wylie, D. R. W. (2004). A dissociation of motion and spatial-pattern vision in the avian telencephalon: Implications for the evolution of "visual streams". *Journal of*

Neuroscience, *24*(21), 4962-4970. https://doi.org/10.1523/JNEUROSCI.0146-04.2004

Olkowicz, S., Kocourek, M., Lučan, R. K., Porteš, M., Fitch, W. T., Herculano-Houzel, S., & Němec, P. (2016). Birds have primate-like numbers of neurons in the forebrain. *PNAS*, *113*(26), 7255-7260. https://doi.org/10.1073/pnas.1517131113

Ono, T., Nishino, H., Fukuda, M., Sasaki, K., & Nishijo, H. (1984). Single neuron activity in dorsolateral prefrontal cortex of monkey during operant behaviour sustained by food reward. *Brain Research*, *311*(2), 323-332. https://doi.org/10.1016/0006-8993(84)90095-7

Passingham, R. (1975). Delayed matching after selective prefrontal lesions in monkeys (*Macaca mulatta*). *Brain Research*, *92*(1), 89-102. https://doi.org/10.1016/0006-8993(75)90529-6

Pasternak, T. (1977). Delayed matching performance after visual Wulst lesions in pigeons. *Journal of Comparative and Physiological Psychology*, *91*(3), 472–484. https://dx.doi.org/10.1037/h0077350

Pasternak, T., & Hodos, W. (1977). Intensity difference thresholds after lesions of the visual Wulst in pigeons. *Journal of Comparative and Physiological Psychology*, *91*(3), 485–497. https://doi.org/10.1037/h0077349

Pasternak, T., & Merigan, W. H. (1994). Motion perception following lesions of the superior temporal sulcus in the monkey. *Cerebral Cortex*, *4*(3), 247-259. https://doi.org/10.1093/cercor/4.3.247

Pearce, J. M., Esber, G. R., George, D. N., & Haselgrove, M. (2008). The nature of discrimination learning in pigeons. *Learning and Behavior*, *36*(3), 188-199. https://doi.org/10.3758/LB.36.3.188

Perea, M., & Lupker, S. J. (2004). Can CANISO activate CASINO? Transposed-letter similarity effects with nonadjacent letter positions. *Journal of Memory and Language*, *51*(2), 231-246. https://doi.org/10.1016/j.jml.2004.05.005

Pettigrew, J. D. (1979). Binocular vision processing in the owl's telencephalon. *Proceedings of the Royal Society of London B*, *204*(1157), 435-454. https://doi.org/10.1098/rspb.1979.0038

Premack, D. (1983). The codes of man and beasts. *Behavioural and Brain Sciences*, *6*(1), 125-136. https://doi.org/10.1017/S0140525X00015077

Rainer, G., Asaad, W. F., & Miller, E. K. (1998). Selective representation of relevant information by neurons in the primate prefrontal cortex. *Nature*, *393*(6685), 577-579. https://doi.org/10.1038/31235

Range, F., Aust, U., Steurer, M., & Huber, L. (2008). Visual categorisation of natural stimuli by domestic dogs. *Animal Cognition*, *11*(2), 339-47. https://doi.org/10.1007/s10071-007-0123-2

Rehder, B., & Hoffman, A. B. (2005). Eyetracking and selective attention in category learning. *Cognitive Psychology*, *51*(1), 1-41. https://doi.org/10.1016/j.cogpsych.2004.11.001

Rehkämper, G., & Zilles, K. (1991). Parallel evolution in mammalian and avian brains: comparative cytoarchitectonic and cytochemical analysis. *Cell and Tissue Research*, *263*, 3-28. https://doi.org/10.1007/BF00318396

Reiner, A., Perkel, D. J., Bruce, L. L., Butler, A. B., Csillag, A., Kuenzel, W., Medina, L., Paxinos, G., Shimizu, T., Striedter, G., Wild, M., Ball, G. F., Durand, S., Güntürkün, O., Lee, D. W., Mello, C. V., Powers, A., White, S. A., Hough, G., Kubikova, L., Smulders, T. V., Wada, K., Dugas-Ford, J., Husband, S., Yamamoto, K., Yu, J., Siang, C., & Jarvis, E. D. (2004). Revised nomenclature for avian telencephalon and some related brainstem nuclei. *The Journal of Comparative Neurology*, *473*(3), 377–414. https://doi.org/10.1002/cne.20118

Revzin, A. M. (1969). A specific visual projection area in the hyperstriatum of the pigeon. *Brain Research*, *15*, 246-249. https://doi.org/10.1016/0006-8993(69)90324-2

Rice, G. A., & Robinson, D. O. (1975). The role of bigram frequency in the perception of words and nonwords. *Memory and Cognition*, *3*(5), 513-518. https://doi.org/10.3758/BF03197523

Riley, N. M., Hodos, W., & Pasternak, T. (1988). Effects of serial lesions of telencephalic components of the visual system in pigeons. *Visual Neuroscience*, *1*(4), 387-394. https://doi.org/10.1017/s0952523800004156

Roberts, W. A., & Mazmanian, D. S. (1988). Concept learning at different levels of abstraction by pigeons, monkeys, and people. *Journal of Experimental Psychology: Animal Behavior Processes*, *14*(3), 247-260. https://doi.org/10.1037/0097-7403.14.3.247

Rogers, L. J. (2008). Development and function of lateralization in the avian brain. *Brain Research Bulletin*, *76*(3), 235-244. https://doi.org/10.1016/j.brainresbull.2008.02.001

Rose, J., & Colombo, M. (2005). Neural correlates of executive control in the avian brain. *PLoS Biology*, *3*(6), e190. https://doi.org/10.1371/journal.pbio.0030190

Rossi, A. F., Pessoa, L., Desimone, R., & Ungerleider, L. G. (2009). The prefrontal cortex and the executive control of attention. *Experimental Brain Research*, *192*(3), 489-497. https://doi.org/10.1007/s00221-008-1642-z

Rothe, J., Schulte-Körne, G., & Ise, E. (2014). Does sensitivity to orthographic regularities influence reading and spelling acquisition? A 1-year prospective study. *Reading and Writing*, *27*(7), 1141-1161. https://doi.org/10.1007.s11145-013-9479-7

Rudolph, K., & Pasternak, T. (1999). Transient and permanent deficits in motion perception after lesions of cortical areas MT and MST in the macaque monkey. *Cerebral Cortex*, *9*(1), 90-100. https://doi.org/10.1093/cercor/9.1.90

Rumelhart, D. E., & Siple, P. (1974). Process of recognizing tachistoscopically presented words. *Psychological Review*, *81*(2), 99-118. https://doi.org/10.1037/h0036117

Scarf, D., Boy, K., Reinert, A. U., Devine, J., Güntürkün, O., & Colombo, M. (2016). Orthographic processing in pigeons (*Columba livia*). *PNAS, 113*(40), 11272-11276. https://doi.org/10.1073/pnas.1607870113

Scarf, D., Hayne, H., & Colombo, M. (2011). Pigeons on par with primates in numerical competence. *Science*, *334*(6063), 1664. https://doi.org/10.1126/science.1213357

Scarf, D., Stuart, M., Johnston, M., & Colombo, M. (2016). Visual response properties of neurons in four areas of the avian pallium. *Journal of Comparative Physiology*, *202*(3), 235-245. https://doi.org/10.1007/s00359-016-1071-6

Schmalz, X., Moll, K., Mulatti, C., & Schulte-Körne, G. (2019). Is statistical learning ability related to reading ability, and if so, why?. *Scientific Studies of Reading*, *23*(1), 64-76. https://doi.org/10.1080.10888438.2018.1482304

Schrier, A. M., & Brady, P. M. (1987). Categorisation of natural stimuli by monkeys (*Macaca mulatta*): Effects of stimulus set size and modification of exemplars. *Journal of Experimental Psychology: Animal Behavior Processes*, *13*(2), 136-143. https://doi.org/10.1037/0097-7403.13.2.136

Seifart, F. (2008). Orthography development. In J. Gippert, N. P. Himmelmann, & U. Mosel (Eds.) *Trends in Linguistics: Essentials of Language Documentation* (pp. 275-300). De Gruyter Mouton.

Seyfarth, R. M., Cheney, D. L., & Marler, P. (1980). Vervet monkey alarm calls: Semantic communication in a free-ranging primate. *Animal Behaviour*, *28*(4), 1070-1094. https://doi.org/10.1016/S0003-3472(80)80097-2

Shimizu, T., & Bowers, A. N. (1999). Visual circuits of the avian telencephalon: evolutionary implications. *Behavioural Brain Research*, *98*(2), 183–191. https://doi.org/10.1016/S0166-4328(98)00083-7

Shimizu, T., Cox, K., & Karten, H. J. (1995). Intratelencephalic projections of the visual Wulst in pigeons (*Columba livia*). *The Journal of Comparative Neurology*, *359*(4), 551-572. https://doi.org/10.1002/cne.903590404

Shimizu, T., & Karten, H. (1993). The avian visual system and the evolution of the neocortex. In H. P. Zeigler & H. J. Bischof (Eds.), *Vision, Brain and Behaviour in Birds* (pp. 103–114). MIT Press.

Shuler, M. G., & Bear, M. F. (2006). Reward timing in the primary visual cortex. *Science, 311*(5767), 1606–1609. https://doi.org/10.1126/science.1123513

Sigala, N., & Logothetis, N. K. (2002). Visual categorisation shapes feature selectivity in the primate temporal cortex. *Nature*, *415*(6869), 318-320. https://doi.org/10.1038/415318a

Smith, E. E., & Grossman, M. (2008). Multiple systems of category learning. *Neuroscience and Biobehavioral Reviews*, *32*(2), 249-264. https://doi.org/10.1016/j.neubiorev.2007.07.009

Smith, J. D., Ashby, F. G., Berg, M. E., Murphy, M. S., Spiering, B., Cook, R. G., & Grace, R. C. (2011). Pigeons' categorisation may be exclusively nonanalytic. *Psychonomic Bulletin and Review*, *18*, 414-421. https://doi.org/10.3758/s13423-010-0047-8

Soto, F. A., & Wasserman, E. A. (2010). Error-driven learning in visual categorisation and object recognition: A Common Elements Model. *Psychological Review*, *117*(2), 349-381. https://doi.org/10.1037/a0018695

Soto, F. A., & Wasserman, E. A. (2012). A category-overshadowing effect in pigeons: Support for the Common Elements Model of object categorisation learning. *Journal of Experimental Psychology: Animal Behavior Processes*, *38*(3), 322-328. https://doi.org/10.1037/a0028803

Stacho, M., Ströckens, F., Xiao, Q., & Güntürkün, O. (2016). Functional organization of telencephalic visual association fields in pigeons. *Behavioural Brain Research*, *303*, 93–102. https://doi.org/10.1016/j.bbr.2016.01.045

Stănişor, L., van der Togt, C., Pennartz, C. M. A., & Roelfsema, P. R. (2013). A unified selection signal for attention and reward in primary visual cortex. *Proceedings of the National Academy of Sciences USA*, *110*(22), 9136–9141. https://doi.org/10.1073/pnas.1300117110

Starosta, S., Güntürkün, O., & Stüttgen, M. C. (2013). Stimulus-response-outcome coding in the pigeon nidopallium caudolaterale. *PLoS ONE*, *8*(2), e57407. https://doi.org/10.1371/journal.pone.0057407

Supèr, H., Spekreijse, H., & Lamme, V. A. F. (2001). A neural correlate of working memory in the monkey primary visual cortex. *Science*, *293*(5527), 120-124. https://doi.org/10.1073/pnas.1300117110

Talbot, S. A., & Marshall, W. H. (1941). Physiological studies on neural mechanisms of visual localization and discrimination. *American Journal of Ophthalmology*, *24*, 1255-1264. https://doi.org/10.1016/S0002-9394(41)91363-6

Tzschentke, T. M. (2000). The medial prefrontal cortex as a part of the brain reward system. *Amino Acids*, *19*(1), 211-219. https://doi.org/10.1007/s007260070051

Valenti, A., Sovrano, V. A., Zucca, P., & Vallortigara, G. (2003). Visual lateralisation in quails (*Coturnix coturnix*). *Laterality*, *8*(1), 67-78. https://doi.org/10.1080/713754470

Vallortigara, G. (2000). Comparative neuropsychology of the dual brain: A stroll through animals' left and right perceptual worlds. *Brain and Language*, *73*(2), 189-219. https://doi.org/10.1006/brln.2000.2303

Vallortigara, G., & Andrew, R. J. (1994). Differential involvement of right and left hemisphere in individual recognition of the domestic chick. *Behavioural Processes*, *33*(1-2), 41-57. https://doi.org/10.1016/0376-6357(94)90059-0

Vaughan, W., & Greene, S. L. (1984). Pigeon visual memory capacity. *Journal of Experimental Psychology: Animal Behavior Processes*, *10*(2), 256-271. https://doi.org/10.1037/0097-7403.10.2.256

Veit, L., Hartmann, K., & Nieder, A. (2014). Neuronal correlates of visual working memory in the corvid endbrain. *The Journal of Neuroscience*, *34*(23), 7778-7786. https://doi.org/10.1523/JNEUROSCI.0612-14.2014

Veit, L., & Nieder, A. (2013). Abstract rule neurons in the endbrain support intelligent behaviour in corvid songbirds. *Nature Communications*, *4*, 2878. https://doi.org/10.1038/ncomms3878

Veit, L., Pidpruzhnykova, G., & Nieder, A. (2017). Learning recruits neurons representing previously established associations in the corvid endbrain. *Journal of Cognitive Neuroscience*, *29*(10), 1712-1724. https://doi.org/10.1162/jocn_a_01152

Verhaal, J., Kirsch, J. A., Vlachos, I., Manns, M., & Güntürkün, O. (2012). Lateralized reward- related visual discrimination in the avian entopallium. *European Journal of Neuroscience*, *35*(8), 1337-1343. https://doi.org/10.1111/j.1460-9568.2012.08049.x

Vogels, R. Categorisation of complex visual images by rhesus monkeys: Part 1: Behavioural study. *European Journal of Neuroscience*, *11*(4), 1223-1238. https://doi.org/10.1046/j.1460-9568.1999.00530.x

von Fersen, L., & Güntürkün, O. (1990). Visual memory lateralization in pigeons. *Neuropsychologia*, *28*(1), 1-7. https://doi.org/10.1016/0028-3932(90)90081-X

von Fersen, L., Wynne, C. D., Delius, J. D., & Staddon, J. E. (1991). Transitive inference formation in pigeons. *Journal of Experimental Psychology: Animal Behaviour Processes*, *17*(3), 334-341. https://doi.org/10.1037/0097-7403.17.3.334

Vonk, J., Jett, S. E., & Mosteller, K. W. (2012). Concept formation in American black bears, *Ursus americanus*. *Animal Behaviour*, *84*(4), 953-964. https://doi.org/10.1016/j.anbehav.2012.07.020

Vonk, J., Jett, S. E., Mosteller, K. W., & Galvan, M. (2013). Natural category discrimination in chimpanzees (*Pan troglodytes*) at three levels of abstraction. *Learning and Behavior*, *41*, 271-284. https://doi.org/10.3758/s13420-013-0103-0

Walsh, V., Carden, D., Butler, S. R., & Kulikowski, J. J. (1993). The effects of V4 lesions on the visual abilities of macaques: hue discrimination and colour constancy. *Behavioural Brain Research*, *53*(1-2), 51-62. https://doi.org/10.1016/S0166-4328(05)80265-7

Wang, B., Wu, P., Kwan, B., Tu, X. M., & Feng, C. (2018). Simpson's paradox: Examples. *Shanghai Archives of Psychiatry*, *30*(2), 139-143. https://doi.org/10.11919/j.issn.1002-0829.218026

Wasserman, E. A., Brooks, D. I., & McMurray, B. (2015). Pigeons acquire multiple categories in parallel via associative learning: A parallel to human word learning?. *Cognition*, *136*, 99-122. https://doi.org/10.1016/j.cognition.2014.11.020

Wasserman, E. A., Castro, L., & Freeman, J. H. (2012). Same-different categorisation in rats. *Learning and Memory*, *19*(4), 142-145. https://doi.org/10.1101/lm.025437.111

Wasserman, E. A., Kiedinger, R. E., & Bhatt, R. S. (1988). Conceptual behaviour in pigeons: Categories, subcategories, and pseudocategories. *Journal of Experimental Psychology: Animal Behaviour Processes*, *14*(3), 235-246. https://doi.org/10.1037/0097/7403.14.3.235

Watanabe, S. (1991). Effects of ectostriatal lesions on natural concept, pseudoconcept, and artificial pattern discrimination in pigeons. *Visual Neuroscience*, *6*(5), 497-506. https://doi.org/10.1017/S0952523800001346

Watanabe, S. (1992). Effect of lesions in the ectostriatum and Wulst on species and individual discrimination in pigeons. *Behavioural Brain Research*, *49*(2), 197–203. https://doi.org/10.1016/S0166-4328(05)80165-2

Watanabe, S. (1993). Effects of ectostriatal lesions, Wulst lesions, and hemispherectomy upon visual discrimination of food in pigeons. *Japanese Journal of Physiological Psychology and Psychophysiology*, *11*(1), 13-22. https://doi.org/10.5674/jjppp1983.11.13

Watanabe, S. (1996). Effects of ectostriatal lesions on discriminations of conspecific, species and familiar objects in pigeons. *Behavioural Brian Research, 81*(1-2), 183-188. https://doi.org/10.1016/S0166-4328(96)89079-6

Watanabe, S. (2003). Effects of Wulst and ectostriatum lesions on repeated acquisition of spatial discrimination in pigeons. *Cognitive Brain Research*, *17*(2), 286–292. https://doi.org/10.1016/S0926-6410(03)00129-0

Watanabe, S, Mayer, U., & Bischof, H.-J. (2011). Visual Wulst analyses "where" and entopallium analyses "what" in the zebra finch visual system. *Behavioural Brain Research*, *222*(1), 51–56. https://doi.org/10.1016/j.bbr.2011.03.035

Watanabe, S., Sakamoto, J., & Wakita, M. (1995). Pigeons' discrimination of paintings by Monet and Picasso. *Journal of the Experimental Analysis of Behavior*, *63*(2), 165–174. https://doi.org/10.1901/jeab.1995.63-165

Wills, A. J., Larvic, A., Croft, G. S., & Hodgson, T. L. (2007). Predictive learning, prediction, errors, and attention: Evidence from event-related potentials and eye tracking. *Journal of Cognitive Neuroscience*, *19*(5), 843-854. https://doi.org/10.1162/jocn.2007.19.5.843

Yamazaki, Y., Aust, U., Huber, L., Hausmann, M., & Güntürkün, O. (2007). Lateralized cognition: Asymmetrical and complementary strategies of pigeons during discrimination of the "human concept". *Cognition*, *104*(2), 315-344. https://doi.org/10.1016/j.cognition.2006.07.004

Zhang, S., Srinivasan, M. V., Zhu, H., & Wong, J. (2004). Grouping of visual objects by honeybees. *Journal of Experimental Biology*, *207*(19), 3289-3298. https://doi.org/10.1242/jeb.01155

Ziegler, J. C., Hannagan, T., Dufau, S., Montant, M., Fagot, J., Grainger, J. (2013). Transposed-letter effects reveal orthographic processing in baboons. *Psychological Science*, *24*(8), 1609-1611. https://doi.org/10.1177/0956797612474322